FINAL REPORT

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GRANT TITLE: Role of P- and E- Selectins in Allergic Inflammation

AWARD PERIOD: 1 February 1996 - 31 January 1998

OBJECTIVE: To attempt to identify and quantify the importance of P-selectin and E-selectin in the pathogenesis of certain features of acute or chronic cutaneous allergic inflammation in the mouse, including tissue swelling, augmented vascular permeability, leukocyte infiltration, proliferation of resident keratinocytes, fibroblasts and vascular endothelial cells.

APPROACH: Quantify features of IgE- and mast cell-dependent cutaneous allergic inflammation in mice that genetically lack P-selectin, E-selectin, or both selectins and in the corresponding control (+/+ or "wild type") mice.

ACCOMPLISHMENTS (throughout the award period): We first established the model system for analyzing IgE- and mast cell-dependent cutaneous allergic reactions in wild type (normal) mice of the correct genetic background (129/Sv x C57BL/6 mice), since the details of the methods needed for optimal analysis of such reactions can vary according to mouse strain. We then analyzed multiple features of IgE- and mast cell-dependent cutaneous reactions in mice that genetically lack P-selectin (P -/-), E-selectin (E -/-), or both of these selectins (P/E -/-) to investigate the possible roles of the selectins in the pathogenesis of these reactions. We found that a lack of either or both selectins had little or no effect on the extent of mast cell degranulation or the tissue swelling associated with these reactions. Moreover, a lack of either P- or E-selectin alone did not reduce the leukocyte (primarily, neutrophil) infiltration at the reaction sites. However, mice lacking both P- and E-selectins exhibited an almost complete ablation of IgE- and mast cell-dependent neutrophil recruitment. These findings, which show that P- and E-selectins can express overlapping functions in leukocyte recruitment associated with IgE- and mast cell-dependent cutaneous late phase reactions in mouse skin, were published in *Laboratory Investigation* in April, 1998 (publication 1 on the attached list).

In addition, we worked on the establishment of reliable methods for analyzing additional aspects of IgE- and mast cell-dependent allergic inflammatory reactions in mouse skin, including assessment of the proliferation of resident cells (keratinocytes, fibroblasts, and vascular endothelial cells) in these reactions and began efforts to establish a model of chronic allergic inflammation in the mouse respiratory tract which could be used to analyze the roles of P- and E-selectins in the pathogenesis of allergic reactions at this site. The latter projects have so far been quite successful, in that reliable model systems have been established for use in normal mice. However, we have not yet analyzed the ability of P- and/or E-selectin -/- mice to express all of the various aspects of these reactions.

CONCLUSIONS: Work derived from many groups, in various experimental animal species, indicates that the particular adhesive interactions which are critical for leukocyte

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13. ABSTRACT *(Maximum 200 words)*

Critical progress has been made in the identification and characterization of cells and mediators involved in allergic inflammation. Accumulating evidence supports the importance of cell adhesion molecule expression as an initiating process in tissue inflammation. Despite progress made to date, much is still unknown about the exact mechanisms responsible for this inflammatory response. Scientists have been working to understand the selective cell recruitment operating in allergic disease with the hope of discovering therapeutic intervention strategies that will prevent the accumulation of unwanted cells in inflamed airways. Research has been directed at developing various approaches to generate specific antagonists. Some approaches under study interupt airway inflammation in its early stages during leukocyte-endothelial interactions. Other approaches inhibit cell recruitment at the endothelial wall. Many studies have been done, both in vivo and in vitro, and the advances that have been made suggest that these therapeutic interventions may be the keys to controlling and, possibly, curing asthma and allergic reactions.

14. SUBJECT TERMS

cell adhesion, allergic rhinitis, atopic allergic conditions, cell biology

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recruitment can vary according to the type of inflammatory stimulus, the vascular bed involved, the time course of the response, and the species or even the strain of experimental animal under investigation. Our results clearly show that in mice (of the 129/Sv x C57BL/6 genetic background), neither P- nor E-selectin (alone or together) are required for expression of the mast cell degranulation and tissue swelling (i.e., vascular permeability changes) that are associated with IgE- and mast cell-dependent allergic reactions in mouse skin. However, mice which genetically lack expression of both P- and E-selectin exhibit almost complete inability to recruit neutrophils at sites of IgE- and mast cell-dependent cutaneous allergic inflammation. By contrast, mice which lack either P- or E-selectin alone exhibit essentially normal neutrophil infiltration at such sites. These findings show that P- and E-selectins can express overlapping functions in leukocyte recruitment associated with IgE- and mast cell-dependent cutaneous late phase reactions in mouse skin, in that a lack of both of these selectins results in virtual elimination of IgE-dependent leukocyte recruitment.

SIGNIFICANCE: Our study, although performed exclusively in mice, supports the hypothesis that P- and E-selectins are critical for the expression of IgE-dependent leukocyte recruitment in the skin; leukocyte recruitment is a critical hallmark of late phase reactions expressed at this and other anatomical sites. Moreover, this set of findings supports the hypothesis that therapeutic agents directed at the inhibition of P- and E-selectin-dependent adhesive interactions might have value as agents to reduce leukocyte infiltration in late phase reactions. However, our results also clearly show that agents which are highly specific in their ability to inhibit selectin-dependent adhesive interactions (for example, agents which target solely P- or E-selectin) may not be effective therapeutically, since these two selectins can exhibit overlapping function in the mediation of leukocyte recruitment.

PATENT INFORMATION: No patent applications related to this proposal have been submitted. No such applications are anticipated.

AWARD INFORMATION: During the period of support, the principal investigator received the following awards (related to his studies of mast cell biology and the role of mast cells in the expression of immunological and inflammatory responses): MERIT Award from the NIAID/NIH (for AI/CA23990, "Regulation of mast cell development and function"); the Paul Kallós Memorial Lecture Award at the 21st Symposium of the Collegium Internationale Allergologicum, Salzburg Austria, September 9, 1996; election to the Association of American Physicians; and the 1997 Scientific Achievement Award of the International Association of Allergy & Clinical Immunology.

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REVIEWS/CHAPTERS:

2. Galli, S.J. (1997) The Paul Kallós Memorial Lecture: The mast cell: A versatile effector cell for a challenging world. Int. Arch. Allergy Immunol. 113:14-22.

3. Galli, S.J. (1997) Complexity and redundancy in the pathogenesis of asthma: Reassessing the roles of mast cells and T cells. J. Exp. Med. 186:343-7.

4. Costa, J.J., Weller, P.F., Galli, S.J. The cells of the allergic response: Mast cells, basophils and eosinophils. In: Baker, J.R. Jr. ed. Primer on Allergic Diseases-Fourth Edition. J. Am. Med. Assoc. 1997; 278:1815-22.

5. Galli, S.J., Lantz, C.S. Allergy. In: Paul, W.E., ed. Fundamental Immunology, 4th edition. Philadelphia, Lippincott-Raven Press, 1999:1137-84.

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P- and E-Selectins Are Required for the Leukocyte Recruitment, but not the Tissue Swelling, Associated with IgE- and Mast Cell-Dependent Inflammation in Mouse Skin

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SUMMARY: Many studies, in both experimental animal and human systems, have indicated that P- and/or E-selectins may contribute importantly to the leukocyte recruitment that occurs in association with mast cell-dependent inflammatory responses. We used mice that genetically lack P-selectin (P −/−), E-selectin (E −/−), or both selectins (P/E −/−) to investigate the possible roles of these selectins in the IgE- and mast cell-dependent recruitment of neutrophils to the skin of mice. We found that a lack of either or both selectins had little or no effect on the extent of mast cell degranulation or the tissue swelling associated with these reactions. Moreover, a lack of either P- or E-selectin alone did not reduce the neutrophil infiltration at the reaction sites. However, mice lacking both P- and E-selectins exhibited an almost complete ablation of IgE- and mast cell-dependent neutrophil recruitment. These findings show that P- and E-selectins can express overlapping functions in leukocyte recruitment associated with IgE- and mast cell-dependent cutaneous late-phase reactions in mouse skin, and that a lack of both selectins results in a virtual elimination of IgE-dependent leukocyte recruitment. (*Lab Invest 1998, 78:497–505*).

The recruitment of circulating leukocytes to local sites of inflammation is mediated by a series of adhesive events that can be orchestrated by many different inflammatory mediators. The basic steps in this process include the tethering of leukocytes to, and their rolling along, activated venular endothelial cells; the activation of the leukocytes by cytokines, chemokines, and/or chemoattractants; the firm adherence of leukocytes to the endothelium via interactions between integrins and members of the immunoglobulin superfamily; and the subsequent emigration of leukocytes out of the vessels into the interstitial tissue (Butcher, 1991; Springer, 1995).

The first step in leukocyte recruitment, which is the rolling of leukocytes along activated venular endothelial cells, is mediated primarily by the selectins or, in some cases, integrins (Butcher, 1991; Springer, 1995). Accordingly, deficiencies in selectins can result in abnormalities in leukocyte recruitment. For example, in several models of acute or chronic inflammation, P-selectin −/− mice exhibit significant impairment of leukocyte recruitment (Bullard et al, 1996; Frenette et al, 1996; Labow et al, 1994; Mayadas et al, 1993; Staite et al, 1996; Subramaniam et al, 1995; Tang et al, 1996), which often is compromised further in mice that lack both P- and E-selectins (Bullard et al, 1996; Frenette et al, 1996; Staite et al, 1996; Tang et al, 1996), whereas E-selectin −/− mice may exhibit little or no abnormalities in leukocyte recruitment in the same settings (Bullard et al, 1996; Labow et al, 1994; Staite et al, 1996). However, studies in rats indicate that leukocyte rolling in certain types of inflammation may occur by selectin- and α_4-integrin–independent mechanisms (Johnston et al, 1997). Taken together, these and other findings (Arbones et al, 1994) show that the particular adhesive interactions that are critical for leukocyte recruitment can vary according to the type of inflammatory stimulus, the vascular bed involved, the time course of the response, and the species or even the strain (Ramos et al, 1997) of the experimental animal.

Upon IgE-dependent activation (Galli, 1993), mast cells release mediators that can enhance the local expression of both P-selectin (ie, histamine and serotonin [Gaboury et al, 1995; Galli, 1993; Jones et al,

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1993; Thorlacius et al, 1994] and TNF-α [Bischoff and Brasel, 1995; Galli, 1993; Gordon and Galli, 1990; Gotsch et al, 1994]) and E-selectin (ie, TNF-α; Galli, 1993; Klein et al, 1989; Leung et al, 1991; Walsh et al, 1991). In addition, a large body of work, from both animal models and human studies, has implicated E- and/or P-selectins in the inflammation associated with various examples of mast cell-dependent or allergic inflammation (Bochner and Schleimer, 1994; Galli and Costa, 1995; Gundel et al, 1991; Klein et al, 1989; Leung et al, 1991; Lukacs et al, 1995; Montefort et al, 1994; Walsh et al, 1991).

However, there have been only two reports describing the expression of allergic inflammation in P- selectin– and/or E-selectin–deficient mice, and both of these studies were conducted with mice that had been actively immunized to ovalbumin (OVA). One study showed that P-selectin was critical for the majority of antigen-induced rolling of leukocytes in the venules of the cremaster muscle, but that P-selectin −/− mice nevertheless exhibited normal levels of leukocyte emigration after antigen challenge (Kanwar et al, 1997). Although no abnormalities in antigen-induced leukocyte rolling or emigration were detected in E-selectin −/− mice, Kanwar et al (1997) reported that mice lacking both P- and E-selectins exhibited no antigen-induced leukocyte emigration. By contrast, in a study of OVA-induced allergic inflammation in mice that had been repetitively challenged by aerosolized antigen over 7 days, P-selectin −/− mice exhibited an approximately 85% reduction in leukocyte counts, versus wild-type mice, in bronchoalveolar lavage fluid after antigen challenge (De Sanctis et al, 1997). Taken together, these studies indicate that role of P-selectin in influencing the extent of leukocyte recruitment during allergic inflammation may vary, from virtually essential (as in the respiratory tract model; De Sanctis et al, 1997) to redundant with E-selectin (as in the cremaster muscle; Kanwar et al, 1997).

In the present study, we used mice genetically deficient in P-, E-, or P- and E-selectins to investigate the contribution of these selectins to the cutaneous leukocyte recruitment that occurs in response to IgE-induced activation of dermal mast cells at sites of passive cutaneous anaphylaxis (PCA). This system is particularly well suited to analyze the role of P- and/or E-selectin in IgE- and mast cell-dependent inflammation. We previously showed that both the tissue swelling (Wershil et al, 1987, 1991) and the leukocyte recruitment in this model is entirely mast cell-dependent and that at least 50% of this leukocyte recruitment is TNF-α–dependent (Wershil et al, 1991). Both mast cell activation (Gaboury et al, 1995; Jones et al, 1993; Klein et al, 1989; Leung et al, 1991; Thorlacius et al, 1994; Walsh et al, 1991) and TNF-α (Bischoff and Brasel, 1995; Gotsch et al, 1994; Klein et al, 1989; Leung et al, 1991; Walsh et al, 1991) can induce enhanced expression of P-selectin (Bischoff and Brasel, 1995; Gaboury et al, 1995; Gotsch et al, 1994; Jones et al, 1993; Thorlacius et al, 1994;) and/or E-selectin (Klein et al, 1989; Leung et al, 1991; Walsh et al, 1991). Moreover, in human tissues, mast cell activation can result in a marked enhancement of E-selectin expression on vascular endothelial cells, a process that is, at least in part, also TNF-α–dependent (Klein et al, 1989; Leung et al, 1991; Walsh et al, 1991). This model also permits the quantitative assessment of two important aspects of allergic inflammation which can be much more difficult to evaluate at other anatomical sites, and which were not analyzed in the prior studies of allergic inflammation in selectin-deficient mice: the extent of mast cell degranulation at such sites and the magnitude of the associated tissue swelling (Wershil et al, 1987, 1991).

Results and Discussion

No Impairment in Tissue Swelling in PCA in P-Selectin and/or E-selectin −/− Mice

As shown in Figure 1, tissue swelling developed rapidly after antigen challenge at sites of PCA reactions (left ears) in all three types of selectin-deficient mice studied, but not at the contralateral control sites (right ears), which had been injected with vehicle rather than IgE. The ear thicknesses in the IgE-injected sites were consistently highest at 1 hour after antigen challenge, at which time the values were significantly greater than those in the contralateral vehicle-injected ears ($p < 0.05$ to 0.001 for all comparisons). In the experiments that were terminated at 6 hours after antigen challenge, there were no statistically significant (ie, $p < 0.05$) differences in the extent of the peak immediate swelling response (at 1 hour) between any of the three selectin −/− mouse groups and the corresponding wild-type (+/+) controls (Fig. 1, A to C). In the one experiment with P/E −/− versus +/+ mice that was terminated at 12 hours after antigen challenge, the tissue swelling associated with the PCA reaction at 1 or 2 hours after antigen challenge was actually somewhat *greater* in the P/E −/− mice than in the +/+ controls ($p < 0.05$ at both intervals, Fig. 1D). The biologic significance of this finding, which occurred in only one of six experiments comparing reactions in P/E −/− versus +/+ mice, remains uncertain.

No Impairment in Mast Cell Degranulation in PCA in Selectin-Deficient Mice

There were no significant differences between numbers of mast cells/mm^2 of ear dermis in any of the selectin −/− and corresponding +/+ mice (data not shown). In addition, more than 60% of the mast cells present in the IgE-injected (left) ears of all groups of selectin −/− or wild-type mice exhibited either moderate or extensive degranulation, and there were no statistically significant differences in the extent of mast cell degranulation at PCA reaction sites in selectin −/− versus corresponding +/+ mice (Fig. 2). By contrast, < 10% of the mast cells present in the vehicle-injected (control) ears exhibited evidence of extensive degranulation and the great majority of the cells appeared normal. For mice of any genotype, the extent of mast cell degranulation at PCA reaction sites

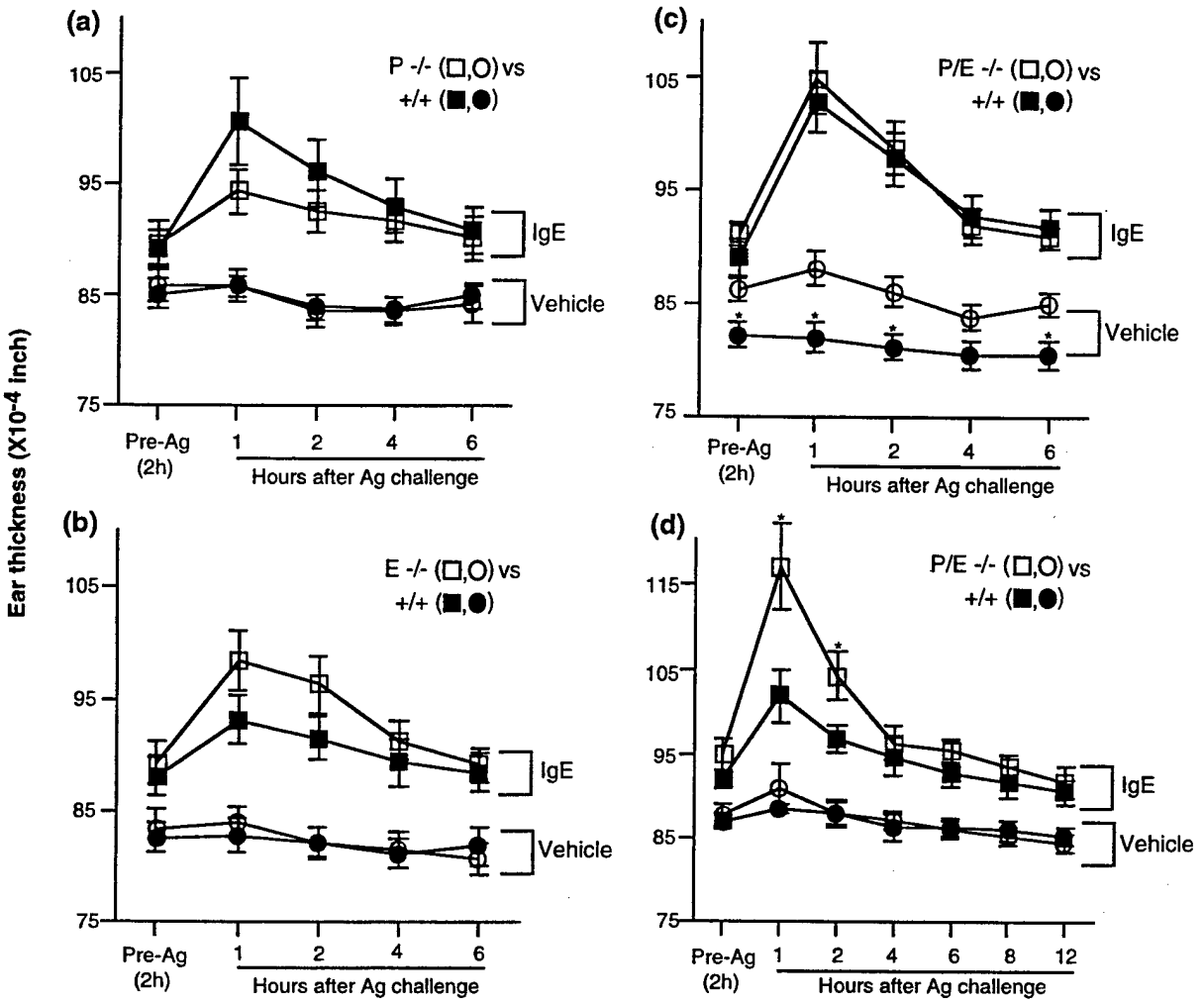

Figure 1.

Ear swelling in IgE-dependent passive cutaneous anaphylaxis (PCA) reactions in mice lacking P-selectin −/− (P −/−) (a), E-selectin −/− (E −/−) (b), or P- and E-selectin −/− (P/E −/−) mice (c and d) and corresponding wild-type (+/+) mice (a to d). The swelling responses were measured by assessing ear thickness at the PCA sites (IgE-injected left ears) and at control sites (vehicle-injected right ears), both before injection of specific antigen (Pre-Ag) and at intervals up to 6 hours (a to c) or 12 hours (d) after intravenous injection of the specific antigen (DNP-HSA). * = $p < 0.05$ versus values for the corresponding wild-type (+/+) mice. The differences in values for IgE-injected and contralateral vehicle-injected ears at 1 hour after antigen injection were statistically significant ($p < 0.001$) in each group of −/− or +/+ mice ($p < 0.001$ in a and b; $p < 0.05$ in c and d).

was significantly greater than that in the contralateral control sites ($p < 0.0001$ for all comparisons).

Impairment of Neutrophil Infiltration in PCA in P/E −/− Mice, but Not in P −/− or E −/− Mice

We previously showed that the intravenous injection of DNP_{30-40}-human serum albumin (DNP-HSA) results in the development of a "late-phase reaction" in IgE-sensitized ears, which is characterized by a peak of PMN infiltration that occurs between 6 and 12 hours after antigen challenge (Wershil et al, 1991). Moreover, approximately 50% of the neutrophil recruitment detected at sites of IgE-dependent PCA reactions at 6 hours was inhibitable with a neutralizing antibody to TNF-α (Wershil et al, 1991). Frenette et al (1996) showed that the ability of TNF-α to enhance leukocyte rolling on TNF-α-treated mesenteric venules was diminished, relative to the striking effect observed in wild-type mice, by approximately 57% in P −/− mice, and was essentially ablated in P/E −/− mice (Frenette et al, 1996). Taken together, these findings suggest that mast cell-dependent neutrophil recruitment to PCA reaction sites might be reduced in P −/− mice and even more markedly impaired in P/E −/− mice.

As shown in Figure 3, a significant enhancement of PMN recruitment was detectable by 6 hours after antigen challenge in the IgE-injected as opposed to the vehicle-injected ears of the wild-type (+/+) mice. However, this was also observed in the P −/− or E −/− mice. Indeed, the extent of PMN infiltration was virtually identical at PCA reaction sites in P −/− versus +/+ mice and in E −/− versus +/+ mice (Fig. 3, A and B). By contrast, in comparison to the results in the +/+ controls, PMN infiltration at IgE-injected sites was markedly reduced in P/E −/− mice that were killed at 6 hours after antigen challenge. The

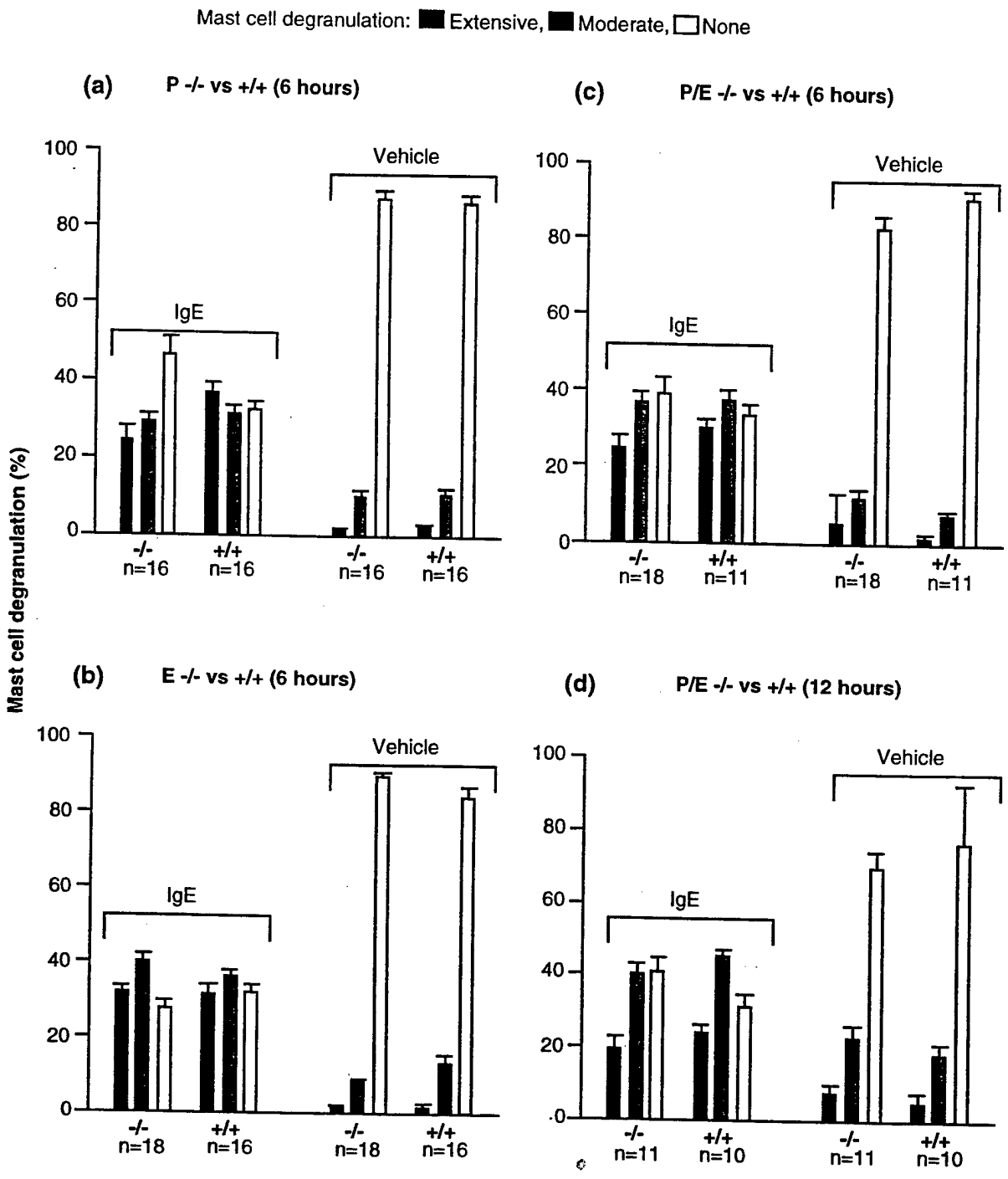

Figure 2.

Extent of degranulation of dermal mast cells at IgE-dependent passive cutaneous anaphylaxis (PCA) reaction sites (IgE) and contralateral control, vehicle-injected sites (vehicle) in the same selectin −/− and corresponding control (+/+) mice shown in Figure 1. Mast cells were classified morphologically as extensively or moderately degranulated or as not degranulated ("none"). There were no statistically significant differences (ie, $p < 0.05$) between any of the results for each type of knockout mouse and the corresponding wild-type mouse. For each type of selectin −/− or corresponding +/+ mouse, the extent of mast cell degranulation in the IgE-injected ears was significantly greater than that in the contralateral control (vehicle-injected) ears ($p < 0.0001$) by the χ^2 test.

mean level of PMN infiltration at PCA sites was only slightly (~70%), albeit significantly, higher than that at the contralateral vehicle-injected sites ($p \cong 0.035$, Fig. 3C), whereas PMN infiltration at sites of IgE-injection in the P/E −/− mice at 12 hours after antigen challenge was virtually identical to that in the contralateral control sites ($p \cong 0.9$, Fig. 3D). Similarly, in an additional experiment with seven P/E −/− mice and seven control mice that were killed at 24 hours after antigen challenge, both the tissue swelling and the PMN infiltration had almost entirely subsided at PCA reaction sites, and there were no statistically significant differences in the values for the P/E −/− mice and the wild-type mice (data not shown). Note that the small

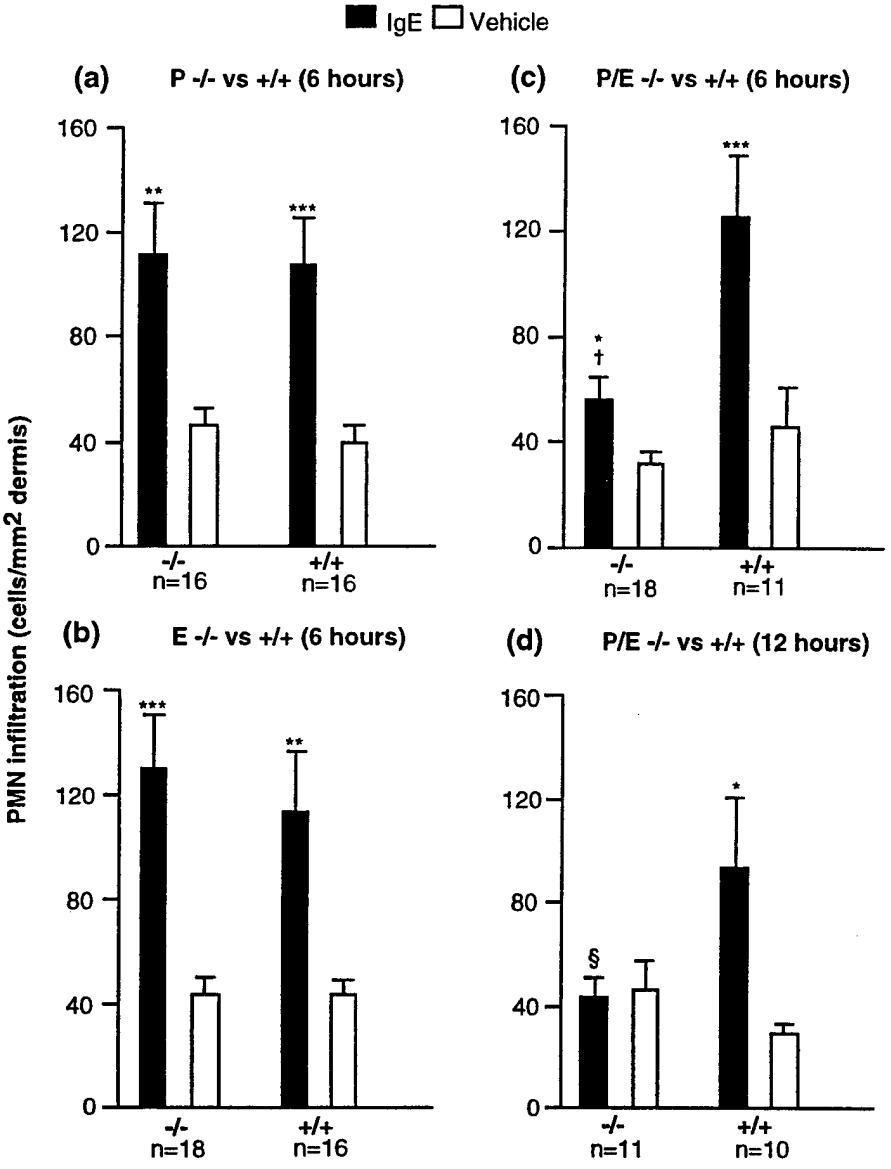

Figure 3.

Neutrophil infiltration into the dermis at 6 hours (a to c) or 12 hours (d) after antigen challenge in the IgE-dependent passive cutaneous anaphylaxis (PCA) reaction sites (IgE) and contralateral control, vehicle-injected sites (vehicle) in the same selectin −/− and corresponding control (+/+) mice shown in Figures 1 and 2. Values for selectin −/− and wild-type mice were compared by the unpaired Student's *t* test; values for IgE-injected versus vehicle-injected ears were compared by the paired Student's *t* test *, **, or *** = $p < 0.04$, ≤ 0.01, or ≤ 0.001, respectively, versus values for the contralateral vehicle-injected ears; § or † = $p \cong 0.08$ or 0.003 versus corresponding values for IgE-injected ears of wild-type (+/+) mice.

numbers of PMN in vehicle-injected control ears, which probably reflected a response to the trauma of the intradermal injection of vehicle, did not differ significantly in the various selectin −/− and corresponding +/+ mice.

We then performed two more experiments to compare PMN infiltration at 6 hours in PCA reaction sites in P/E −/− versus +/+ mice, but this time we used a dilution of ascites containing a monoclonal mouse anti-DNP IgE (Liu et al, 1980; Wershil et al, 1987, 1991) to sensitize left ears, and injected the right ears with the diluent (HMEM/Pipes) alone. In both experiments, the P/E −/− and +/+ mice did not differ in the magnitude of the mast cell degranulation or tissue swelling at the PCA or control sites (data not shown), but the P/E −/− mice exhibited levels of PMN recruitment to PCA reaction sites that were significantly less than those in the PCA reactions sites in the wild-type mice, yet not significantly different from those in the contralateral vehicle-injected ears (Fig. 4).

The negligible levels of leukocyte recruitment to sites of IgE-dependent mast cell degranulation in P/E −/− mice do not reflect diminished levels of circulating leukocytes. Indeed, in confirmation of our previous report (Frenette et al, 1996), the P/E −/− mice used in these experiments had significantly elevated levels of peripheral blood leukocytes, compared with the +/+ mice, both at baseline and at 6 hours after the elicita-

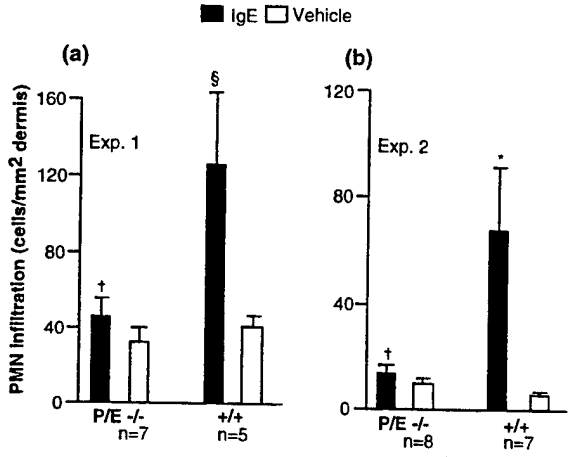

Figure 4.

Neutrophil infiltration into the dermis at 6 hours after antigen challenge in the IgE-dependent PCA reaction sites (IgE) and contralateral control, vehicle-injected (Vehicle) sites in 2 different experiments (a and b) with five to eight P/E −/− and corresponding wild-type (+/+) mice that had been injected with a dilution of ascites containing a mouse monoclonal IgE–anti-DNP antibody. Values for selectin −/− and wild-type mice were compared by the unpaired Student's *t* test; values for IgE-injected versus vehicle-injected ears were compared by the paired Student's *t* test. § or * = $p = 0.104$ or 0.040 versus values for the contralateral vehicle-injected ears; † = $p < 0.04$ versus corresponding values for IgE-injected ears of wild-type (+/+) mice.

tion of a PCA reaction in one ear (Fig. 5). Note that both P/E −/− and wild-type mice exhibited significantly elevated numbers of blood leukocytes 6 hours after the elicitation of PCA reactions.

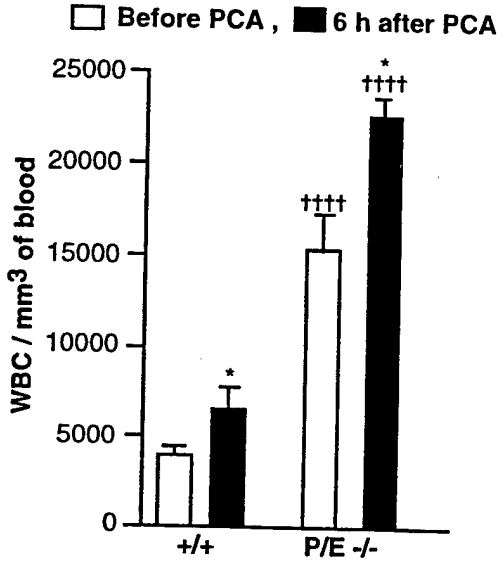

Figure 5.

White blood cells (WBC) per mm^3 of peripheral blood in P/E −/− and corresponding wild-type (+/+) mice 1 to 2 days before and 6 hours after intravenous injection of DNP-HSA to elicit IgE-dependent PCA reactions in the left ears. Data are from the same mice whose results are depicted in the "d" sections of Figures 1 to 3. Values for P/E −/− versus +/+ mice were compared by the unpaired Student's *t* test; values obtained before versus 6 hours after induction of PCA were compared by the paired Student's *t* test. * = $p < 0.05$ versus baseline (before PCA) values for mice of that genotype; †††† = $p < 0.0001$ versus corresponding (before PCA or 6 hours after PCA) values for wild-type (+/+) mice.

Conclusions

Much of the pathology associated with allergic inflammation is thought to reflect the activities of the leukocytes that are recruited to the sites of the allergic reactions (Bochner and Schleimer, 1994; Galli, 1993; Galli and Costa, 1995; Gundel et al, 1991; Lemanske and Kaliner, 1993; Lukacs et al, 1995; Montefort et al, 1994). We have shown that an absence of either P- or E-selectin alone had no significant effect on the levels of neutrophil recruitment that developed over 6 hours at sites of IgE- and mast cell-dependent inflammation elicited in the skin of 129/Sv × C57BL/6 mice. However, such leukocyte recruitment was markedly diminished when both selectins were missing. Thus, P- and E-selectins express critical but overlapping roles in this entirely IgE- and mast cell-dependent model of neutrophil recruitment to mouse skin.

P- and E-selectins also appear to exhibit essential, but largely overlapping, roles in leukocyte recruitment to sites of allergic inflammation elicited in the cremaster muscle of 129/Sv × C57BL/6 mice that were actively immunized to OVA (Kanwar et al, 1997). Although the latter example of allergic inflammation has not yet been formally tested to assess the extent to which it is mast cell-dependent, passive transfer experiments with heat-treated or untreated serum indicate that the inflammation in this model is probably largely dependent on IgE (Kanwar et al, 1997). Taken together with our findings, this study thus supports the conclusion that, in mice, the IgE- and mast cell-dependent recruitment of leukocytes to sites challenged with a single injection of antigen is much more markedly impaired in the absence of both P- and E-selectins than in the absence of only one of these selectins. Indeed, in both of these models of allergic inflammation, a lack of P- or E-selectin alone had no detectable effect on the extent of antigen-induced leukocyte recruitment. By contrast, a lack of P-selectin alone has been reported to be associated with a marked impairment in the leukocyte accumulation at sites of repetitive antigen challenge of the respiratory tract in mice that have been actively immunized to OVA (De Sanctis et al, 1997). It remains to be determined, however, whether the apparent differences in the importance of P-selectin for leukocyte recruitment in these three models of allergic inflammation are due to differences in the anatomical sites at which the reactions were elicited, the effects of single versus multiple administrations of antigen, and/or other factors. For example, much of the leukocyte recruitment that is elicited by antigen challenge in the respiratory tract of actively immunized mice can occur by mast cell-independent mechanisms (Brusselle et al, 1994; Coyle et al, 1996; Galli, 1997; Kung et al, 1995; Nogami et al, 1990; Takeda et al, 1997).

Products of recruited neutrophils can contribute to both mast cell activation (Molin and Stendahl, 1984) and enhanced vascular permeability (Wedmore and Williams, 1981; Williams et al, 1984), and the augmentation in vascular permeability that was measured at 4 hours after the elicitation of *Streptococcus*

pneumoniae-induced peritonitis (Bullard et al, 1996) or during cytokine-induced meningitis (Tang et al, 1996), was significantly reduced in P −/− mice and even more greatly diminished in P/E −/− mice. Although tissue swelling at sites of 24-hour cutaneous contact hypersensitivity reactions was unaffected in P-selectin −/− mice (Staite et al, 1996; Subramaniam et al, 1995) or E-selectin −/− mice (Staite et al, 1996), the swelling response was significantly reduced (by ~50% versus wild-type or E- or P-selectin −/− mice) in P- and E-selectin −/− mice (Staite et al, 1996). However, in contrast to these other models of inflammation, the leukocyte recruitment that develops at sites of IgE-dependent PCA reactions occurs primarily after much of the tissue swelling has waned (Wershil et al, 1991). In accord with the kinetics of leukocyte recruitment to such reactions, we found that both the mast cell degranulation and the tissue swelling at IgE-dependent PCA reactions appeared to occur independently of any important role for P- and E-selectins, or P- and/or E-selectin-dependent leukocyte recruitment.

Materials and Methods

Selectin-Deficient Mice

The generation, by gene targeting in embryonic stem cells, of three strains of mice that genetically lack either P-selectin (P −/−) (Mayadas et al, 1993), E-selectin (E −/−) (Frenette et al, 1996), or both P- and E-selectins (P/E −/−) (Frenette et al, 1996), and the phenotypic characteristics of these mice, have been described in detail. All of the mice used for these experiments, including the wild-type (+/+) controls, were 7- to 9-week–old females on the (129/Sv × C57BL/6) genetic background.

Elicitation of IgE-Dependent Passive Cutaneous Anaphylaxis and Late-Phase Reactions

Each mouse was primed to express an IgE-dependent passive cutaneous anaphylaxis (PCA) reaction in the left ear, and a control reaction in the contralateral (right) ear, as described in detail elsewhere (Wershil et al, 1991). Briefly, mice were sensitized in the left ear by an intradermal injection of 100 ng of monoclonal mouse dinitrophenyl-specific (anti-DNP) IgE (Sigma Chemical Company, St. Louis, Missouri) in 20 μl of Hanks' minimal essential medium (HMEM; GIBCO Laboratories, Grand Island, New York) containing 0.47 g/l Pipes buffer instead of $NaCO_3$ (HMEM/Pipes), whereas the right (control) ear received an intradermal injection of 20 μl of vehicle (HMEM/Pipes) alone. The next day (at "time 0"), the mice received an intravenous injection of 1000 μg of DNP-HSA (Sigma) diluted in phosphate-buffered saline (PBS) at pH 7.4. Groups of six to nine selectin-deficient mice and wild-type controls were used in 2 to 3 replicate experiments for mice of each selectin −/− genotype. In some experiments, peripheral blood was obtained (for white blood counts) from the retro-orbital sinus of mice that had been anesthetized with ether.

Assessment of Tissue Swelling, Leukocyte Recruitment, and Mast Cell Degranulation

Ear swelling was evaluated by measuring the ear thickness (in units of 10^{-4} inch) with a micrometer (Wershil et al, 1987, 1991) 2 hours before (Pre-Ag = Pre-antigen) and at 1, 2, 4, 6, and 12 hours after the administration of DNP-HSA.

We previously reported that the majority of cells (> 90%) that infiltrated sites of IgE- and mast cell-dependent cutaneous inflammation were neutrophils (PMN), and that maximal levels of PMN infiltration occurred between 6 and 12 hours after antigen challenge (Wershil et al, 1991). In the present experiments, the mice were killed by CO_2 inhalation at 6 or 12 hours after antigen challenge; tissue from the PCA reaction sites (left ears) and the contralateral control sites (right ears) were processed into 1-μm, Epon-embedded, Giemsa-stained sections, which were coded and then examined by light microscopy by one observer who was unaware of the identity of the individual sections (Wershil et al, 1991). The mast cells were classified as extensively degranulated (> 50% of the cytoplasmic granules exhibiting fusion, staining alterations, and/or extrusion from the cell), moderately degranulated (10% to 50% of the granules exhibiting such changes), or normal (Wershil et al, 1991). Using a computer-assisted morphometric approach, the numbers of mast cells and PMN present at the PCA or control reaction sites were counted and the results were expressed as the number of cells per mm^2 of dermis (Wershil et al, 1991).

Statistical Analysis

Results are expressed as mean ± SEM The paired Student's *t* test was used to compare responses in IgE-injected (left) and vehicle-injected (right) ears, whereas the unpaired Student's *t* test was used to compare reactions in the selectin −/− mice and corresponding wild-type mice. The χ^2 test was used to compare results for extent of mast cell degranulation. Replicate experiments in mice of the same genotype yielded very similar results; accordingly, the results were pooled according to genotype for presentation in the figures.

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Chapter 2

The Cells of the Allergic Response

Mast Cells, Basophils, and Eosinophils

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Mast cells, basophils, and eosinophils have long been regarded as important effector cells in allergic disorders. Indeed, it is thought that the cells' cytoplasmic granule–associated or lipid mediators contribute to many of the signs and symptoms that are characteristic of these diseases. Mast cells, basophils, and eosinophils also probably contribute to protective host responses, especially to parasites. In addition, recent evidence shows that mast cells, basophils, and eosinophils can secrete a wide spectrum of cytokines and, in some cases, express functions that may permit them to regulate the development or perpetuation of allergic responses. Thus, mast cells, basophils, and eosinophils may express immunoregulatory activities, as well as serve as effector cells.

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THIS CHAPTER will review the general biology, products, and functions of mast cells, basophils, and eosinophils, focusing particularly on the roles of these cells in allergic diseases. Because of space limitations, we have elected to rely heavily on the citation of recent reviews, rather than original reports, as references for further reading.

MAST CELLS AND BASOPHILS

While mast cells and basophils share several notable features, they are distinct cell types (Table 2-1). Both mast cells and basophils contain cytoplasmic granules that have the distinctive property of exhibiting metachromasia when stained with certain basic dyes. Both cell types are derived from bone marrow progenitor cells, and both mast cells and basophils are major sources of histamine and other potent chemical mediators (Table 2-2) that have been implicated in a wide variety of inflammatory and immunologic processes, including allergic disorders with components of immediate hypersensitivity.[1-4] In all mammalian species yet analyzed, both mast cells and basophils constitutively express plasma membrane receptors that specifically bind with high affinity the Fc portion of the IgE antibody (FcεRI).[5,6]

Although it once was believed that basophils might be circulating precursors of mast cells or that mast cells were "tissue basophils," current evidence indicates that mature basophils are terminally differentiated circulating granulocytes that can infiltrate tissues or appear in exudates during a variety of inflammatory or immunologic processes. By contrast, morphologically identifiable mature mast cells do not normally circulate but instead mature within the vascularized tissues in which they reside.

Morphology and Development

Morphologic Features.—Routine methods of tissue fixation and processing are poorly suited for demonstration of basophils and mast cells; optimal visualization is achieved in appropriately prepared, 1-μm plastic sections or with an ultrastructural approach.[1] Ultrastructurally, human basophils typically exhibit a segmented nucleus with marked condensation of nuclear chromatin and contain round or oval cytoplasmic granules (Figure 2-1). By contrast, mast cells typically appear as either round or elongated cells with a nonsegmented or, occasionally, bilobed or multilobed nucleus with moderate condensation of nuclear chromatin and contain cytoplasmic granules that are usually smaller, more numerous, and generally more variable in appearance than those in basophils (Figure 2-1).

Production of Mast Cells and Basophils.—Like other granulocytes, basophils are derived from pluripotent CD34+ hematopoietic progenitor cells, ordinarily differentiate and mature in the bone marrow and then circulate in the blood.[3,4] Interleukin 3 (IL-3) appears to be an important developmental factor for basophils, although other growth factors may also influence basophil development.[3,4] The basophil is the least common blood granulocyte in humans, with a prevalence of approximately 0.5% of total leukocytes and approximately 0.3% of nucleated marrow cells. While human basophils appear to exhibit kinetics of production and peripheral circulation similar to those of eosinophils, unlike the eosinophil, the basophil ordinarily does not occur in peripheral tissues in significant numbers.[1,3,4] Basophils can infiltrate sites of many immunologic or inflammatory processes, often in association with eosinophils, and also can participate in the reactions to some tumors.

Mast cells are also derived from CD34+ hematopoietic progenitor cells.[2-4] However, except for the small numbers of mast cells that are resident in the bone marrow, mast cell maturation typically occurs in the peripheral tissues. Several lines of evidence indicate that interactions between the tyrosine kinase receptor c-kit, which is expressed on the surface of mast cells and their precursors, and the c-kit ligand, stem cell factor (SCF), are essential for normal mast cell development and survival.[7] For example, mice with mutations that result in either markedly impaired c-kit function or a marked reduction in the expression of membrane-associated SCF virtually lack tissue mast cells,[7] and subcutaneous administration of recombinant human SCF can induce mast cell hyperplasia in vivo in humans.[8] Stem cell factor is expressed on the plasma membrane of a variety of cell types, including fibroblasts, bone marrow stromal cells, and vascular endothelial cells, and the extracellular domain of SCF can be released from the cells by proteolytic cleavage; both the membrane-associated and soluble forms of SCF are biologically active.[7] It is likely that local levels of SCF regulate mast cell numbers in normal tissues and also contribute to the striking alterations in mast cell numbers noted in association with a variety of immunologic reactions, reparative responses, and disease processes.[7-13] However, it is also likely that the development and phenotypic characteristics of human mast cell populations can be influenced by a complex interplay of cytokines and growth factors other than SCF.[7-11]

Mast Cell Distribution and Heterogeneity.—Mast cells are distributed throughout normal connective tissues,

Table 2-1.—Natural History of Mast Cells, Basophils, and Eosinophils

Characteristic	Mast Cells	Basophils	Eosinophils
Origin of precursor cells	$CD34^+$ hematopoietic progenitor cells	$CD34^+$ hematopoietic progenitor cells	$CD34^+$ hematopoietic progenitor cells
Site of maturation	Connective tissue (a few in bone marrow)	Bone marrow	Bone marrow
Mature cells in circulation	No	Yes (usually <1.0% of blood leukocytes)	Yes
Mature cells recruited into tissues from circulation	No	Yes	Yes
Mature cells normally residing in connective tissues	Yes (numbers can increase greatly at sites of chronic inflammation)	No	Yes
Proliferative ability of mature cells	Yes (under certain circumstances)	No	No
Life span	Weeks to months (according to studies in rodents)	Days	Days to weeks
Major developmental factor	Stem cell factor	Interleukin 3	Interleukin 5
Expression of FcεRI*	High levels constitutively and regulated by IgE	High levels constitutively and regulated by IgE	Low levels†

*FcεRI indicates plasma membrane receptors that specifically bind with high affinity the Fc portion of IgE antibody.
†Can be detected on some eosinophils; the role of IgE in regulation of FcεRI expression in eosinophils is not yet clear.

Table 2-2.—Products of Mast Cells, Basophils, and Eosinophils*

Mediators	Mast Cells	Basophils	Eosinophils
Major mediators stored preformed in cytoplasmic granules	Histamine, heparin and/or chondroitin sulfates, neutral proteases (tryptase with or without chymase), many acid hydrolases, cathepsin G, carboxypeptidase	Histamine, chondroitin sulfates, neutral protease with bradykinin-generating activity, β-glucuronidase, elastase, cathepsin G–like enzyme, major basic protein, lysophospholipase (Charcot-Leyden crystal protein)	Major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, eosinophil peroxidase, lysosomal hydrolases, lysophospholipase (Charcot-Leyden crystal protein)
Major lipid mediators produced on appropriate activation	Prostaglandin D_2, leukotriene C_4, platelet-activating factor	Leukotriene C_4	Leukotriene C_4, lipoxins
Cytokines†	IL-4, IL-5, IL-6, IL-8, IL-13, TNF-α, MIP-1α, bFGF	IL-4, IL-13	IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-16, GM-CSF, TNF-α, RANTES, MIP-1α, eotaxin, TGF-α, TGF-β_1, VEGF/VPF

*IL indicates interleukin; TNF-α, tumor necrosis factor α; MIP-1α, macrophage inflammatory protein 1α; bFGF, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; TGF, transforming growth factor; and VEGF/VPF, vascular endothelial cell growth factor/vascular permeability factor.
†In some cases, the listings are based on immunohistochemical evidence indicating that at least some cells of this type contain immunoreactivity for the cytokine, rather than proof that the biologically active cytokine can be released from the cell.

where they often lie adjacent to blood and lymphatic vessels, near or within nerves, and beneath epithelial surfaces that are exposed to the external environment, such as those of the respiratory and gastrointestinal systems and the skin.[7,9-13] Mast cells are also a normal if numerically minor component of the bone marrow and lymphoid tissues. However, unlike mature basophils, mature mast cells do not normally circulate in the blood.

In humans and many other mammalian species, mast cell numbers in normal tissues exhibit considerable variation according to anatomic site, and these baseline numbers of mast cells can change in association with certain inflammatory or immunologic reactions.[7,9-13] For example, the numbers of mast cells at sites of chronic inflammation due to a variety of different causes may well be many times higher than in the corresponding normal tissues.[7,9-13]

The concept of mast cell heterogeneity is based on evidence derived from studies in humans and experimental animals that indicate that mast cells can vary in many aspects of phenotype, including morphology, histochemistry, mediator content, and response to drugs and stimuli of activation.[7,9-13] Although the regulation and functional significance of mast cell heterogeneity remain to be fully defined, it is likely that phenotypically distinct mast cell populations may express different functions in health and disease.[7,9-13]

Mediators

Basophils and mast cells contain, or elaborate on appropriate stimulation, a diverse array of potent biologically active mediators (Table 2-2).[1,3,4,11,14-18] Some of these products are stored preformed in the cells' cytoplasmic granules (eg, proteoglycans, proteases, histamine); others are synthesized on activation of the cell by IgE and antigen or other stimuli (eg, products of arachidonic acid oxidation through the cyclooxygenase or lipoxygenase pathways and, in some cells, platelet activating factor). Cytokines are the most recently identified group of mast cell and basophil mediators, at least one of which, tumor necrosis factor α (TNF-α), can be both preformed and stored in mast cells, as well as newly synthesized by activated cells.[15]

Preformed Mediators.—Mediators stored preformed in the cytoplasmic granules include histamine, proteoglycans, serine proteases, carboxypeptidase A, and small amounts of sulfatases and exoglycosidases.[1,3,4] Mast cells and basophils form histamine by the decarboxylation of histidine. Studies in genetically mast cell–deficient and congenic normal mice indicate that mast cells account for nearly all the histamine stored in normal tissues, with the exception of the glandular stomach and the central nervous system. Basophils are the source of most of the histamine in normal human blood.

Human mast cell populations contain variable mixtures of heparin (about 60 kd) and chondroitin sulfate proteoglycans.[3,11,14,16] Although the sulfated glycosaminoglycans of normal human blood basophils have not been characterized, chondroitin sulfates account for the majority of the proteoglycans in the basophils of patients with myelogenous leukemia. Mast cell and basophil proteoglycans probably have several biologic functions both within and outside the cells. By ionic interactions they bind histamine, neutral proteases, and carboxypeptidases, and they may contribute to the packaging and storage of these molecules within the secretory granules. When the granule matrices are exposed to physiologic conditions of pH and ionic strength during degranulation, the various mediators associated with the proteoglycans dissociate at different rates, histamine very rapidly but tryptase and chymase much more slowly.[3] In addition to regulating the kinetics of release of mediators from the granule matrices, proteoglycans can also regulate the ac-

tivity of some of the associated mediators (see below).

Neutral proteases are the major protein component of mast cell secretory granules. Both basophils and mast cells contain enzymes with tosyl-L-arginine methyl esterase (TAME)–esterase activity, which can be used as a marker of mast cell or basophil activation in vivo. By weight, tryptase is the major enzyme stored in the cytoplasmic granules of human mast cells, and this neutral protease occurs in most, if not all, human mast cell populations.[3] Human mast cell tryptase is a serine endopeptidase that exists in the granule in active form as a tetramer of 134 kd that contains subunits of 31 to 35 kd, each of which contains an active site. Negligible amounts of tryptase have been identified in normal human basophils by immunoassay. Because this enzyme appears to be highly characteristic of, if not unique to, the human mast cell, measurements of mast cell tryptase in biologic fluids, such as plasma, serum, and inflammatory exudates, have been used to assess mast cell activation in these settings. Tryptase is stored in the cytoplasmic granules in the active tetrameric form as a complex that is stabilized by its association with heparin and perhaps other proteoglycans within the mast cell granule. The function of mast cell tryptase in vivo is unknown. Mast cell chymase is also a serine protease that is stored in active form in the granules of some, but not all, human mast cells, but as a monomer with a molecular weight of 30 kd. Human basophils, like eosinophils, can form Charcot-Leyden crystals and contain Charcot-Leyden crystal protein (lysophospholipase) in quantities similar to those of eosinophils.[3]

Newly Synthesized Lipid Mediators.—The activation of mast cells or basophils with appropriate stimuli not only causes the secretion of preformed granule-associated mediators, but also can initiate the de novo synthesis of certain lipid-derived substances. Of particular importance are the cyclooxygenase and lipoxygenase metabolites of arachidonic acid, which have potent inflammatory activities and which may also play a role in modulating the release process itself.[16,18] The major cyclooxygenase product of mast cells is prostaglandin D_2 (PGD_2), and the major lipoxygenase products derived from mast cells and basophils are the sulfidopeptide leukotrienes (LT): LTC_4 and its peptidolytic derivatives, LTD_4 and LTE_4. Human mast cells, but not human basophils, also can produce LTB_4, albeit in much smaller quantities than PGD_2 or LTC_4.[16,18]

There are at least 3 patterns of release of products of arachidonic acid metabolism by human mast cells and basophils[14]: (1) Gut or lung mast cells produce similar amounts of LTC_4 and PGD_2. (2) Skin mast cells produce largely PGD_2. (3) Basophils generate primarily LTC_4.

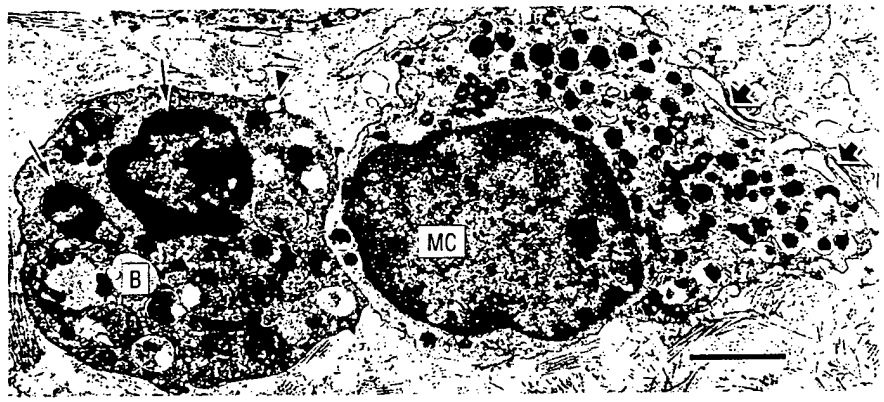

Figure 2-1.—A basophil (B) adjacent to a mast cell (MC) in the ileal submucosa of a patient with Crohn disease. The basophil exhibits a bilobed nucleus (solid arrows), whose chromatin is strikingly condensed beneath the nuclear membrane. The basophil surface is relatively smooth with a few blunt processes (arrowhead). The mast cell nucleus is larger and its chromatin less condensed than that of the basophil. The mast cell's granules are smaller, more numerous, and more variable in shape and content than those of the basophil. The mast cell surface has numerous elongated, thin folds (open arrows). (Bar=2 μm.) (Modified with permission from Dvorak AM, Monahan RA, Osage JE, Dickersin GR. Crohn's disease: transmission electron microscopic studies, II: immunologic inflammatory response: alterations of mast cells, basophils, eosinophils, and the microvasculature. *Hum Pathol.* 1980;11:606-619.)

Cytokines.—Cytokines are a diverse group of glycoproteins that are synthesized and, typically, secreted by many cell types in response to their activation or injury; cytokines can modulate both specific immune responses and immunologically nonspecific inflammation (and other biologic processes) through their ability to alter the function or gene expression of responsive target cells. Many cytokine-dependent processes are implicated in allergic inflammation, including the up-regulation of the IgE response itself (eg, IL-4, IL-13); the enhancement or induction of basophil recruitment (eg, TNF-α, IL-4) or mediator production (eg, IL-3, IL-4, the C-C chemokine, macrophage inflammatory protein [MIP]–1α); the promotion of eosinophil development survival (eg, IL-5, IL-3, granulocyte-macrophage colony-stimulating factor [GM-CSF]) and recruitment (eg, IL-3, IL-5, IL-16, GM-CSF, certain C-C chemokines); and the recruitment of monocytes and T cells (eg, IL-16, certain C-C chemokines).[17] Much of the ability of certain cytokines (ie, IL-1, TNF-α) to promote allergic inflammation is thought to reflect the ability of these agents to enhance the recruitment of leukocytes by inducing the increased expression of adhesion molecules, such as P-selectin and E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1, on vascular endothelial cells.[17,19,20] However, cytokines may critically influence many other stages in the development of allergic inflammation, as well as regulate some of the local consequences of these responses.

It is now clear that mast cells and basophils have the potential to influence many important aspects of the pathogenesis of allergic inflammation in asthma and other allergic disorders via the elaboration of cytokines.[15,21] Indeed, the production of cytokines appears to represent one of the critical links between IgE-dependent mast cell activation that occurs immediately after allergen challenge in atopic subjects, the inflammation that develops during the subsequent late-phase reactions (LPRs) to such provocation, and the persistent inflammation and associated tissue changes that are characteristic of chronic allergic disorders.[21] For example, studies in mast cell–reconstituted, genetically mast cell–deficient mice have demonstrated that mast cells are required for essentially all the leukocyte infiltration observed in the skin or stomach wall after challenge with IgE and specific antigen.[21-23] A similar approach was used to show that mast cells can contribute to the eosinophil infiltration elicited in the lungs in response to aerosol allergen challenge in sensitized mice.[24] In the skin, approximately 50% of such IgE- and mast cell–dependent leukocyte infiltration can be inhibited using an antibody to recombinant mouse TNF-α.[21,22]

In IgE-dependent reactions, mast cells are likely to represent one critical initial source of TNF-α. Other cellular elements of allergic inflammation also can produce this cytokine, such as macrophages, T cells, and B cells, but these cells apparently contain little or no preformed TNF-α bioactivity. By contrast, certain mature, "resting" (nonactivated) mast cells contain preformed stores of TNF-α available for immediate release on appropriate stimulation of the cells.[15,17]

Mast cells represent a potential source of many cytokines, in addition to TNF-α, that might influence allergic inflammation, and the synthesis and release of these products can be induced via IgE-dependent mechanisms. Thus, certain mouse mast cells activated via the FcεRI contain increased levels of messenger RNA for many cytokines (IL-1α, IL-3, IL-4, IL-5, IL-6, and GM-CSF and MIP-1α, MIP-1β, and several other C-C chemokines) and secrete substances with the corresponding bioactivities (IL-1, IL-3, IL-4, IL-6, IL-9, GM-CSF, and IL-13).[15,17,21,25,26] In part because of difficulty obtaining highly purified preparations of human mast cells, studies of human mast cell cytokine production have been slow to emerge. However, human mast cells also appear to represent a potential source of many cytokines, including TNF-α, basic fibroblast growth factor, IL-4, IL-5, IL-6, IL-8, and IL-13.[25]

Glucocorticoids can inhibit cytokine production in many cell types, as can cyclosporine, and these effects have been proposed as one of the important mechanisms of action of these agents in patients with asthma.[27] In mice, both glucocorticoids and cyclosporine can diminish mast cell cytokine production in vitro and can also suppress mast cell– and TNF-α–dependent allergic inflammation in vivo.[28]

Although the ability of basophils to produce cytokines has been less extensively studied than mast cell cytokine production, several reports have demonstrated that mature human basophils isolated from peripheral blood can release IL-4 and IL-13 in response to FcεRI-dependent activation, and that such release can be enhanced in basophils exposed to IL-3 but not to certain other cytokines.[1,3,4] It is possible that IL-4, IL-13, and/or MIP-1α derived from mast cells or basophils at sites of allergic inflammation may play a role in enhancing IgE production or driving T-cell differentiation toward a functionally distinct subset (of the T_H2 phenotype), whose pattern of cytokine production promotes the allergic response. Moreover, recent findings indicate that human basophils and mast cells also express the CD40 ligand and thus may be able to contribute to IgE production by promoting immunoglobulin class switching.[1,17,29]

Mechanisms of Mast Cell or Basophil Activation

FcεRI-Mediated Activation.—The best understood cellular event that underlies expression of basophil or mast cell function is degranulation, a stereotyped constellation of stimulus-activated biochemical and morphologic events that result in the fusion of the cytoplasmic granule membranes with the plasma membrane (with external release of granule-associated mediators). Although a variety of agents can initiate basophil or mast cell degranulation, the best studied pathway of stimulation is transduced through FcεRI expressed on the basophil or mast cell surface.[5,6] The FcεRI consists of 1 α chain (which binds the Fc portion of IgE), 1 β chain (which functions as an amplifier of signaling via the FcεRI), and 2 identical disulfide-linked γ chains (which are the main intracellular signaling elements of the receptors)[5,6,30]; all 3 chains have been cloned and sequenced.[5,6] When adjacent FcεRIs are bridged, either by bivalent or multivalent antigens interacting with receptor-bound IgE or by antibodies directed against either receptor-bound IgE or the receptor itself, the cells are rapidly activated for the release of stored and newly generated mediators. This process is energy and temperature dependent; requires the mobilization of calcium, which results in increased levels of free calcium in the cytosol; and occurs without evidence of toxic effects to the responding cell. It has been shown that the bridging of only a few hundred pairs of IgE molecules is sufficient to trigger human basophil histamine release.[31]

Because so few of a basophil's or mast cell's FcεRI must be bridged to initiate the degranulation response, these cells may be sensitized simultaneously with IgE antibodies of many different specificities and therefore can react to stimulation by many different antigens. IgE- and antigen-dependent activation is the basis for the immunologically specific expression of mast and basophil function in IgE-dependent immune responses and allergic disorders.

Regulation of IgE Receptor Expression on Mast Cells and Basophils.—In 1977, 2 groups independently demonstrated that the level of FcεRI expression on circulating human basophils can exhibit a positive correlation with the serum concentration of IgE.[32,33] However, the basis for this association was not determined. It has recently been reported that exposure to IgE results in a striking up-regulation of surface expression of FcεRI on mouse or human mast cells in vitro or in mouse mast cells or basophils in vivo.[34,35] In addition, baseline levels of FcεRI expression on peritoneal mast cells and bone marrow basophils from genetically IgE-deficient mice were dramatically reduced (by ≥80%) compared with those on cells from corresponding normal mice. IgE-dependent up-regulation of FcεRI expression in turn significantly enhanced the ability of mouse or human mast cells to release preformed mediators and cytokines in response to challenge with IgE and specific antigen or anti-IgE.[34] Conversely, treatment of allergic subjects with anti-IgE reduced basophil FcεRI expression and, in some cases, levels of FcεRI-dependent secretion of histamine by basophils.[36]

The findings that IgE can be a major regulator of mast cell and basophil FcεRI surface expression in vivo and that IgE-dependent enhancement of mast cell FcεRI expression permits mast cells to respond to antigen challenge with increased production of proinflammatory and immunoregulatory mediators identify a potentially important mechanism for enhancing the expression of effector cell function in IgE-dependent allergic reactions or immunologic responses to parasites.

Nonimmunologic Direct Activation or Modulation of Mast Cell or Basophil Activation.—In addition to IgE and specific antigen, a variety of biologic substances, including products of complement activation and certain cytokines, chemical agents, and physical stimuli, can elicit release of basophil or mast cell mediators.[1,3,12-14,16] However, the responsiveness of human basophils and different populations of human mast cells to individual stimuli varies. For example, cutaneous mast cells appear to be much more sensitive to stimulation by neuropeptides or morphine than are pulmonary mast cells.[12-14,16] Moreover, these stimuli can induce a pattern of mediator release that differs from the one associated with FcεRI-dependent mast cell activation. A considerable body of evidence indicates that certain cytokines can directly activate mast cells or basophils and/or modulate the mediator release from these cells in response to IgE and antigen or other stimuli. However, the effects of individual cytokines are often markedly different in mast cells vs basophils.[1,3,4,25]

Function in Health and Disease

Immediate Hypersensitivity.—The immediate hypersensitivity reaction is the pathophysiologic hallmark of allergic rhinitis, allergic asthma, and anaphylaxis, and the central role of the mast cell in the pathogenesis of these disorders is widely accepted. An immediate hypersensitivity reaction is initiated by the interaction of antigen-specific IgE molecules on the surface of mast cells and/or basophils with the relevant multivalent antigen. The physiologic effects are due to the biologic responses of target cells (vascular endothelial cells, smooth muscle, glands, leukocytes, and so on) to mediators released by activated mast cells and/or basophils. Immediate allergic reactions are usually accompanied by

an increase in local levels of LTC_4 and PGD_2 and by the liberation of histamine and tryptase.[3,16] Although there are several possible cellular sources for some of these mediators, tryptase is thought to be largely if not entirely mast cell derived, providing the strongest biochemical evidence implicating mast cells in these responses in humans.

Other stimuli besides allergens, including certain complement fragments (anaphylatoxins), neutrophil lysosomal proteins, a variety of basic peptides and peptide hormones, insect venoms, radiocontrast solutions, cold, calcium ionophores, and certain drugs such as narcotics and muscle relaxants, may also initiate the rapid release of mediators from basophils and mast cells, independently of IgE.[3,16] The clinical reactions provoked by these agents can closely mimic those of immediate hypersensitivity.

Late-Phase Reactions.—In many allergic patients the immediate reaction to cutaneous antigenic challenge is followed 4 to 8 hours later by persistent swelling and leukocyte infiltration termed the *LPR*.[37] Late-phase reactions may develop following IgE-dependent reactions in the respiratory tract, nose, and other anatomic locations as well as on the skin. Moreover, many of the clinically significant consequences of IgE-dependent reactions are now thought to reflect the actions of the leukocytes recruited to these sites during LPRs rather than the direct effects of the mediators released by mast cells at early intervals after antigen challenge.[17,25,37] Several lines of evidence derived from both clinical and animal studies indicate that mast cell activation and mediator-cytokine secretion contribute to the leukocyte infiltration associated with LPRs.

In humans, the leukocytes recruited to sites of LPRs include basophils, eosinophils, neutrophils, and macrophages; all these cells may influence the reactions by providing additional proinflammatory mediators and cytokines. The recruitment and activation of basophils at LPR sites are supported by analyses of nasal lavage or bronchoalveolar lavage fluids obtained several hours after antigen challenge, which demonstrate elevations in histamine, TAME-esterase activity, and LTC_4, but not PGD_2 or tryptase.[1,3]

Mast Cell–Leukocyte Cytokine Cascades.—Galli and others[3,15,17,21] have formulated the hypothesis that a "mast cell–leukocyte cytokine cascade" critically contributes to the initiation and perpetuation of IgE-dependent allergic inflammation in the airways and other sites. Specifically, it is proposed that the activation of mast cells through the FcεRI initiates the response, in part through the release of TNF-α and other cytokines that can influence the recruitment and function of additional effector cells. These recruited cells then promote the further progression of the inflammatory response by providing additional sources of certain cytokines (that can also be produced by mast cells stimulated by ongoing exposure to allergen), as well as new sources of cytokines and other mediators that may not be produced by mast cells. Finally, mast cell activation may directly or indirectly promote the release of cytokines from certain resident cells in the respiratory tract, such as alveolar macrophages, bronchial epithelial cells, vascular endothelial cells, fibroblasts, epithelial cells, and nerves; cytokines released in these responses then contribute to the vascular and epithelial changes and to the tissue remodeling, angiogenesis, and fibrosis that are so prominent in many disorders associated with mast cell activation and leukocyte infiltration. At certain points in the natural history of these complex processes, cytokines derived from mast cells, or from eosinophils or other recruited cells, may also contribute to the down-regulation of the response.

In addition to their roles in allergic diseases, mast cell–leukocyte cytokine cascades may contribute to host defense, both in innate immunity to microbial infection, in which mast cells are activated independently of IgE, and in immune responses to parasites.[38]

Parasitic Diseases.—Several lines of evidence indicate that mast cells or basophils may have similar, overlapping, or complementary functions in immune responses to ectoparasites, worms, and perhaps other parasites, with the relative contributions of each cell type varying according to the type of parasite, species of host animal, and other factors. For example, the duration of experimental parasite infections with *Trichinella spiralis* and *Strongyloides ratti* was longer in mast cell–deficient mice than in the corresponding normal mice.[39] However, the impairment of immunity in mast cell–deficient mice was never as severe as in athymic nude mice, and in each instance the mast cell–deficient mice eventually were able to resolve the infection. Moreover, the successful elimination of some parasites in the absence or virtual absence of a specific IgE response has also been reported. Thus, mast cell hyperplasia and activation may contribute to host defense against certain helminthic infections, but in many cases mast cells and IgE may not represent the only important effector mechanism in these immune responses. The most compelling evidence for a role for mast cells or basophils in defense against parasites is in immune responses to ectoparasites such as ticks. However, the relative importance of basophils and mast cells in reactions to ticks appears to vary according to the species of host and the species of tick.[1,40-42]

EOSINOPHILS

Eosinophils, like neutrophils and basophils, are types of bone marrow–derived granulocytes (Table 2-1). The original defining property of eosinophils was the striking affinity of their cytoplasmic granules for acid aniline dyes such as eosin. Eosinophils contain several eosinophil-specific proteins in their cytoplasmic granules, yet to date no cell-surface proteins unique to eosinophils have been recognized. Thus, tinctorial properties and other morphologic characteristics remain the routine basis for identifying and enumerating these leukocytes in blood and tissues. In tissues, eosinophils may be underestimated with routine eosin staining and specific techniques, including immunostaining for eosinophil granule proteins or eosinophil fluorescence after fluorescein isothiocyanate or Giemsa staining, may be needed to fully recognize eosinophils. Eosinophilia, characterized by both heightened production of eosinophils in bone marrow and the accumulation of eosinophils in tissues and blood, is characteristically associated with a spectrum of immune responses or pathologic processes that include allergic disorders, helminthic parasitic infections, and a variety of other diseases with less defined causes.

Morphology and Development

Morphologic Features.—Eosinophils are similar in size to neutrophils but have bilobed nuclei and distinctive cytoplasmic granules.[43] The numerous specific granules, with their structured packaging of cationic proteins (which confer on the cell its "eosinophilia"), are the eosinophil's distinguishing morphologic feature.[43] Ultrastructurally, these specific granules contain characteristic, distinct, usually electron-dense, crystalloid cores (Figure 2-2). Eosinophils also contain 2 other types of granules: primary granules, which lack a crystalloid core and develop early in eosinophil maturation, and smaller granules, which contain arylsulfatase and other enzymes. Prominent tubulovesicular structures are sometimes identified as a fourth population of granules (microgranules).[44] Eosinophils, also contain varying numbers of lipid bodies, which are non–membrane-bound, lipid-rich inclusions that are also found in mast cells and many other types of cells and have roles in the formation of paracrine eicosanoid mediators.[44]

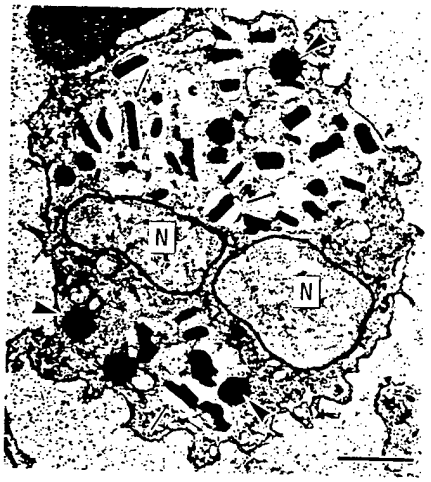

Figure 2-2.—Mature peripheral blood eosinophil from a patient with the idiopathic hypereosinophilic syndrome processed with the reduced osmium technique, osmium potassium ferrocyanide, showing the cells' bilobed nucleus (N), irregular, blunt surface processes, granules, and mitochondria. Dark cytoplasmic particles are monoparticulate glycogen. Four large, round, osmiophilic lipid bodies (open arrowheads) are present. Specific granules are elongated, membrane-bound structures with dense central crystal(s) and lightly dense matrix (arrows). Primary granules do not have central crystals (open arrow). (Bar = 1.4 μm.) (Modified with permission from Dvorak AM, Ackerman SJ, Weller PF. Subcellular morphology and biochemistry of eosinophils. In: Harris JR, ed. *Blood Cell Biochemistry: Megakaryocytes, Platelets, Macrophages and Eosinophils*. London, England: Plenum Publishing Corp; 1991;2:237-344.)

Production of Eosinophils.—Eosinophils are terminally differentiated granulocytes that develop in the bone marrow but reside in large numbers in the peripheral tissues (Table 2-1). Eosinophils are most abundant in tissues with a mucosal epithelial interface with the environment, including the respiratory, gastrointestinal, and lower genitourinary tracts. Although their precise life span is not known, eosinophils live longer than neutrophils and may survive for weeks within tissues.

The development and differentiation of eosinophils are promoted by at least 3 cytokines. Granulocyte-macrophage colony-stimulating factor, IL-3, and IL-5 promote eosinophilopoiesis in vivo, with IL-5 having the most cell-specific effects on eosinophil differentiation and production.[45] Interleukin 5 also acts to rapidly release from the marrow into the circulation a pool of already developed eosinophils.[46] Interleukin 5 is produced by the T_H2-like subset of $CD4^+$ T cells, whereas IL-3 and GM-CSF are elaborated by both T_H1 and T_H2 subsets. Eosinophils also can elaborate each of these cytokines (see below).

Cell-Surface Receptors and Proteins.—Human eosinophils express receptors for IgG (normally FcγRI and FcγRII [CDw32]), IgA (FcαR), and IgE.[47] IgE-binding factors on human eosinophils include the S-type lectin, galectin-3, as well as a low-affinity FcεRII (CD23) and a high-affinity FcεRI.[47] Both CD23 and FcεRI are detectable on eosinophils in sites of allergic reactions, including the airways.[48] The eosinophil receptor for IgA binds secretory IgA more potently than other forms of IgA.[49] Because eosinophils localize to mucosal surfaces of the respiratory, gastrointestinal, and genitourinary tracts, their IgA receptors could engage secretory IgA at these sites.

Eosinophil receptors for complement components include those for C1q, C3b/C4b (CR1), iC3b(CR3), and C5a. Receptors for several cytokines have been identified on eosinophils, including those for IL-3, IL-5, and GM-CSF. Eosinophils express receptors for a number of ligands that are chemoattractants, including platelet activating factor, LTB_4, C5a, and the chemokine eotaxin. These chemoattractants for eosinophils can also stimulate degranulation and the formation of superoxide anion and other oxidant derivatives. In addition, eosinophils express receptors for various other cytokines, including IL-2, IL-4, IL-16 (CD4), and interferon α, and have intracellular receptors for estrogens and glucocorticoids.

In addition to receptors, eosinophils express cell-surface proteins involved in cell-cell interactions. The expression of specific integrins by eosinophils not only may contribute to their preferential recruitment into sites of allergic diseases, but also may help regulate their activation within extravascular tissues.[50] In contrast to neutrophils, eosinophils, like lymphocytes, can emigrate into inflammatory sites in patients with the leukocyte adhesion deficiency syndrome, indicating that eosinophils can migrate into tissues by mechanisms not dependent on CD18. Eosinophils express ligands for binding to both E-selectin and P-selectin.[51] In addition, eosinophils express 2 α_4 integrins. Eosinophils express the $\alpha_4\beta_1$ integrin very late activation antigen 4.[52,53] The very late activation antigen 4 binds to vascular cell adhesion molecule-1 and to domains within tissue fibronectin. By this means, preferential recruitment or activation of eosinophils and mononuclear leukocytes might be expected. The expression of a second α_4 integrin, $\alpha_4\beta_7$, on eosinophils is intriguing, given the role this integrin plays in binding to the mucosal vascular addressin cell adhesion molecule-1 and the enhanced expression of $\alpha_4\beta_7$ on mucosal trophic $CD4^+$ memory T lymphocytes.[54] The common expression by eosinophils and some lymphocyte populations of $\alpha_4\beta_7$ may contribute to their colocalization within lymphoid tissues.

Studies with blocking monoclonal antibodies to the α_4 component of both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ have demonstrated that such blockade can prevent eosinophil influx into cutaneous or pulmonary sites of elicited allergic reactions.[55,56] Moreover, blockade of α_4 integrins can have beneficial effects on allergic reactions, such as inhibiting pulmonary LPRs, even without inhibiting eosinophil influx.[57]

Early eosinophil precursors that develop within the bone marrow express class II major histocompatibility complex proteins, but blood eosinophils, from most normal and eosinophilic donors, lack expression of class II major histocompatibility complex proteins, even if these circulating eosinophils otherwise exhibit phenotypic evidence of in vivo activation. However, when mature, blood-derived human eosinophils are cultured in vitro with specific cytokines, including IL-3, GM-CSF, and interferon γ, these eosinophils are uniformly induced to synthesize and express HLA-DR.[58] Eosinophils in the sputum of patients with asthma have been shown to express HLA-DR, as do airway, but not blood, eosinophils in allergic subjects challenged with antigen via segmental airways or by inhalation or in patients with chronic eosinophilic pneumonia.

Mediators

Intracellular Constituents.—The specific granules contain lysosomal hydrolases as well as the cationic proteins unique to eosinophils (Table 2-2).[59] The crystalloid core of the granule contains major basic protein, and the noncore matrix contains eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase. Major basic protein has no recognized enzymatic activity, but is toxic to helminthic parasites, tumor cells, and host cells.[60] Eosinophil cationic protein, a markedly cationic polypeptide with bactericidal and helminthotoxic activities, like major basic protein, is also toxic to host cells.[60] Eosinophil-derived neurotoxin, a protein that shares some sequence similarity with eosinophil cationic protein, was named not for an established capacity to damage human nerves, but rather because it, like eosinophil cationic protein, induced cerebrocerebellar dysfunction when injected intracerebrally into rabbits.[60] Both proteins have partial sequence identity with pancreatic ribonuclease and have ribonuclease catalytic activity; eosinophil-derived neurotoxin is about 100 times more potent as a ribonuclease than eosinophil cationic protein.[60] Eosinophil peroxidase, an enzyme distinct from the myeloperoxidase of neutrophils and monocytes, consists of 2 polypeptides of about 15 and 55 kd. By using hydrogen peroxide and halide ions, including chloride or, preferentially, bromide, eosinophil peroxidase catalyzes

the formation of hypohalous (eg, hypochlorous or hypobromous) acid. In the presence of hydrogen peroxide and halide ions, eosinophil peroxidase is toxic to helminthic and protozoan parasites, bacteria, tumor cells, and host cells.[60] Another distinctive protein of human eosinophils is the protein that forms Charcot-Leyden crystals (also produced by basophils), the bipyramidal crystals that are often found in sputum, feces, and tissues as a hallmark of eosinophil-related disease. This 17-kd protein, which crystallizes in vivo and in vitro, is a hydrophobic protein with lysophospholipase activity.[61] It comprises about 5% of the eosinophil's total protein, is found in primary granules, and is also associated with cell membranes of eosinophils.

Lipid Mediators.—Lipid mediators formed by eosinophils include platelet activating factor and eicosanoid derivatives of arachidonic acid.[62] Although eosinophils can synthesize prostanoids derived from the cyclooxygenase pathway, the principal eosinophil eicosanoids are products of arachidonate lipoxygenation via both the 5- and 15-lipoxygenase pathways. The predominant product of the 5-lipoxygenase pathway in human eosinophils is LTC_4.[62] The 3 sulfidopeptide leukotrienes, LTC_4 and its derivatives LTD_4 and LTE_4, are potent stimulants of vasoactivity, smooth muscle contraction, and mucous secretion. In addition, eosinophils, unlike neutrophils, contain large quantities of 15-lipoxygenase.[62] Lipoxins, products of double lipoxygenation, and peptide mediators such as substance P are also formed by eosinophils.

Cytokines.—The recognition in recent years that eosinophils represent a potential source of many cytokines has suggested that these cells may influence inflammatory reactions and other biologic responses through a broader spectrum of mechanisms than had previously been supposed.[63,64] Interestingly, many of these eosinophil cytokines appear to be stored within specific granules providing a potential preformed pool of cytokine available for release by eosinophils. The cytokines elaborated by eosinophils may be grouped into 3 categories.[64] The first category, growth factors and chemokines, includes growth factor cytokines, GM-CSF, IL-3, and IL-5, which may exert autocrine and paracrine effects on eosinophils and other cells, serving for eosinophils to enhance their survival and augment their functional responses. Chemokines made by eosinophils include RANTES, MIP-1α, and eotaxin. The second category of cytokines comprises those that may be involved in inflammation, fibrosis, wound healing, and repair, including TGF-α, TGF-β1, vascular endothelial cell growth factor/vascular permeability factor, TNF-α, IL-1α, IL-6, and IL-8. The third category of cytokines comprises those with potential activities in regulating immune responses, such as IL-2, IL-4, IL-10, and IL-16.

Eosinophil Activation

It has long been recognized that eosinophils from eosinophilic donors exhibit metabolic, morphologic, and functional changes indicating that they have been "activated" in vivo.[61] Many of the effector responses (eg, degranulation, eicosanoid formation) of eosinophils can be enhanced by specific eosinophil-active cytokines, including GM-CSF, IL-3, and IL-5. While the eosinophil-active growth factor cytokines contribute to the process of eosinophil "activation," these cytokines alone do not elicit all measures of eosinophil activation,[65] such as enhanced expression of FcεRI[66] or CD40[67] found on eosinophils from allergic subjects. Other cytokines or tissue- or extracellular matrix–derived activating stimuli are also likely to be involved in augmenting specific functional capabilities of eosinophils.

Functions in Health and Disease

Efforts to define the functions of the eosinophil as a distinct class of leukocytes have been pursued ever since the prominence of this cell in various diseases was appreciated. Although many functions have been proposed, evidence now indicates that, as effector cells, eosinophils can have roles that are either beneficial or detrimental to the host.

Host Defense.—Although eosinophils are capable of phagocytosing and killing bacteria and other small microbes in vitro, they probably do not have a major role in host defense against such microbial pathogens in vivo and cannot effectively defend against bacterial infections when neutrophil function is deficient. Rather, eosinophils appear to defend against large, nonphagocytosable organisms, most notably the multicellular helminthic parasites. Based on in vitro studies demonstrating that eosinophils function as helminthotoxic effector cells, it has been hypothesized that a major beneficial function of eosinophils is to participate in host defense against helminthic parasites. This putatively beneficial role is contrasted with some of the deleterious effects of eosinophils identified for allergic diseases. Recent studies with anti–IL-5 antibody–treated, helminth-infected mice, however, have questioned this role, since neutralizing anti–IL-5 antibody has abrogated infection-induced blood, marrow, and tissue eosinophilia, but not the intensities of primary or secondary infections.[68,69] Thus, the nominally beneficial function of eosinophils in parasite host defense remains to be fully validated.

Elicitation of Host-Cell Dysfunction and Damage.—Effector responses of eosinophils also may contribute to the physiologic and pathologic reactions associated with eosinophilia, including immediate hypersensitivity and other allergic diseases. Eosinophil products that are the most damaging to the host are the cationic proteins. Elevated concentrations of such proteins can be detected in the sputum of patients with asthma, in nasal and bronchoalveolar lavage fluid after the experimental inhalation of antigens, and within involved tissues. In allergic diseases, eosinophil granule proteins may cause damage and desquamation of airway epithelial cells, alter airway hyperreactivity and cilial function, and elicit local edema. Moreover, eosinophil-derived eicosanoids, platelet activating factor and LTC_4, may contribute to airway bronchoconstriction and inflammation. Thus, some of the mechanisms used by eosinophils in host defense can also have effects that prove detrimental to the host; the dysfunction and damage caused by eosinophil granule proteins may contribute to the pathogenesis of diseases in which large numbers of eosinophils are found in the involved tissues.

Other Functions.—In addition to effector functions, eosinophils most likely can have other roles in immune responses. For example, the eosinophils that usually reside in normal mucosal tissues probably participate in mucosal immunity, but their specific functions in individual responses at mucosal surfaces have not yet been delineated. Additional roles are possible, but not yet fully defined. Eosinophils probably have roles in wound healing and repair and can be associated with fibrotic disorders. Finally, eosinophils have the capacity to influence other immune responses, including T-lymphocyte–dependent responses. As noted above, eosinophils can be sources of IL-4, IL-2, and IL-16. Moreover, eosinophils may function as antigen-presenting cells, perhaps with roles for the processing and presentation of inhaled and ingested particulate allergens. This role may be particularly important in mucosal tissues exposed to the external environment, sites where eosinophils are abundant.

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Commentary

Complexity and Redundancy in the Pathogenesis of Asthma: Reassessing the Roles of Mast Cells and T Cells

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Asthma affects millions of people worldwide, and its reported incidence is increasing dramatically in many developed nations; the human and economic costs of this disorder, in morbidity, health care expenses, lost productivity, and, most tragically, even mortality, are staggering (1, 2).

It is now generally thought that asthma is a syndrome, typically characterized by the three cardinal features of intermittent and reversible airway obstruction, airway hyperresponsiveness, and airway inflammation, that may arise as a result of interactions between multiple genetic and environmental factors (1–4). Nevertheless, most cases of the disorder (the so-called "atopic" or "allergic" asthma) occur in subjects whom also exhibit immediate hypersensitivity responses to defined environmental allergens, and challenge of the airways of these subjects with such allergens can produce reversible airway obstruction (1–5). It is also known that the overall incidence of asthma in several different populations exhibits a strong positive correlation with serum concentrations of IgE, which, in humans, is the main (if not the only) Ig isotype that can mediate immediate hypersensitivity responses (1, 5). Moreover, it has been demonstrated that mast cells, derivatives of hematopoietic precursor cells that undergo their terminal stages of differentiation/maturation in the peripheral tissues in which they reside (6, 7), express cell surface receptors (FcεRI) that permit them to bind the Fc portion of IgE with high affinity, and also that such IgE-sensitized mast cells, upon encounter with specific antigen that is recognized by their FcεRI-bound IgE, secrete a broad panel of bioactive mediators, including: (*a*) preformed mediators that are stored in the cell's cytoplasmic granules (e.g., histamine, heparin, and neutral proteases), (*b*) newly synthesized lipid products (e.g., prostaglandin D_2 and leukotriene C_4), and (*c*) diverse cytokines (4, 6, 8, 9). Finally, several lines of evidence indicate that many of these potentially mast cell–derived mediators can promote reversible airway obstruction, bronchial hyperreactivity, and/or airway inflammation (see reviews in references 2–4, 8, and 9).

In light of these findings, it was once widely believed that atopic or allergic asthma is a disease that primarily reflects the consequences of IgE- and allergen-dependent mast cell activation. Yet several observations have called into question the central role of mast cells in asthma. These include the demonstration that additional cell types, including eosinophils (10) and Th2 lymphocytes (11), both of which are well represented in the chronic inflammatory infiltrates in the airways of patients with asthma (2–4, 12, 13), also can produce cytokines or other mediators that may contribute to many of the features of the disease. Moreover, it has recently been shown that the FcεRI, which was once thought to be restricted to tissue mast cells and basophils (circulating granulocytes that can produce a panel of mediators that is similar, but not identical, to that of mast cells [6, 9]) can also be expressed on the surface of monocytes, circulating dendritic cells, Langerhans' cells, and eosinophils (see reviewes in references 14 and 15), thus identifying these cells as additional potential sources of mediators in various IgE-dependent inflammatory responses.

Given the large number of potential culprits, some of which can express similar or overlapping functions, how can one assess the relative importance of individual cell types in the pathogenesis of asthma? Although this represents an exceedingly difficult challenge in the setting of human asthma, some aspects of this issue are accessible by taking advantage of animal models of the disease. However, when considering the results of such animal studies, several points should be kept in mind. (*a*) These are models of human asthma, not asthma itself, and the extent to which the findings in these models actually elucidate the human condition(s) needs to be demonstrated by appropriate studies in human subjects. (*b*) Experimental animal species and humans can differ in significant details of immunological and inflammatory responses (e.g., in the mouse, antigen- and mast cell–dependent airway obstruction can be mediated by either IgE or IgG_1 [16], whereas it seems likely that only IgE is involved in the analogous human responses [1, 5]). (*c*) The procedures of allergen sensitization and challenge that are used in animal models of asthma are typically "optimized" to give strong responses for endpoint analysis, and this (as well as the increasing costs of animal experimentation, which discourages the use of large numbers of animals) may make it difficult to detect contributions of cell types that function to amplify the intensity or kinetics of such responses at relatively low levels of allergen challenge. (*d*) Finally, the models used by different investigators may differ in a number of factors, which can have potentially significant effects on the results, including the species (or strain) of experimental animal, the choice of antigen, the protocols for antigen sensitization and challenge, and the means of assessing and quantifying the individual characteristics of the responses.

In other words, the demonstration that a particular cell

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or mediator can produce a feature of asthma in an animal model neither proves that this element can have the same effect in human asthma nor excludes the possibility that other cells or mediators can have similar, and perhaps even more critical, functions, either in experimental animals or humans.

These reservations notwithstanding, how have the results of studies in experimental animals influenced our thinking about the pathogenesis of asthma, and in particular, the potential role of the mast cell in the expression of the three cardinal features of the disorder? The most definitive approach for characterizing the importance of a single potential effector cell or molecule in a biological response is to attempt to elicit the response in animals that differ solely in having or lacking the element of interest. With respect to mast cells, the best current approximation of this ideal is to investigate genetically mast cell–deficient (WB-*W*/+ × C57BL/6-W^v/+)F_1-*W*/W^v (WBB6F_1-*W*/W^v) mice (now more properly designated WBB6F_1-Kit^W/$Kit^{W\text{-}v}$ mice [17]) and the congenic normal (WBB6F_1-+/+) mice (6, 18, 19). Because of the effects of their mutations at c-*kit*, which encodes the receptor for a pleiotropic growth factor that represents a major mast cell survival/developmental factor, stem cell factor (also known as kit ligand or mast cell growth factor; 18), adult Kit^W/$Kit^{W\text{-}v}$ mice virtually lack tissue mast cells (<1.0% the +/+ number in the skin, essentially none in the airways and other sites), but they are also mildly anemic, lack melanocytes in the skin and interstitial cells of Cahal in the gastrointestinal tract, and are sterile due to a virtual absence of germ cells (18–22). However, these mice appear to have little or no abnormalities of B or T cell function, levels of granulocytes (including basophils) or platelets, or hemostasis, nor do they exhibit Ig deficiencies or impairments in their ability to generate IgE or IgG_1 antibody responses (18, 19). Finally, the mast cell deficiency of Kit^W/$Kit^{W\text{-}v}$ mice can be selectively repaired by the adoptive transfer of lineage-committed immature mast cells (BMCMCs, or bone marrow-derived cultured mast cells) which have been generated in vitro from the bone marrow cells of the congenic +/+ mice (6, 19, 21). Such "mast cell knock-in mice" can be used to test whether abnormalities in the expression of biological responses in Kit^W/$Kit^{W\text{-}v}$ mice, which theoretically could be due to any direct or indirect consequence of their c-*kit* mutations, specifically reflect the animals' mast cell deficiency (6, 18, 19, 21).

Studies in Kit^W/$Kit^{W\text{-}v}$ and congenic normal mice have clearly established that, in the mouse: (*a*) IgE-dependent acute reversible airway obstruction can occur by mechanisms that appear to be entirely mast cell dependent (16, 22, 23). (*b*) Although many manifestations of active anaphylactic reactions in the mouse, including changes in airway function and death, can occur by IgE- and mast cell–independent, but IgG_1-dependent, mechanisms, IgE and mast cells probably contribute importantly to the initial rapid and partially reversible phases of airway obstruction, and diminished pulmonary compliance, that are observed during certain models of active anaphylaxis (16). (*c*) The acute airway hyperresponsiveness to intravenous methacholine challenge that can be detected in immunologically naive mice 20 min after intravenous challenge with anti–mouse IgE antibodies is largely (if not entirely) mast cell dependent, and is expressed before the development of any histologically apparent leukocyte infiltration in the airways at sites of mast cell degranulation (23). Although the specific mediator(s) responsible for this example of mast cell–dependent airway hyperreactivity remain to be defined, the candidates include representatives of all three classes of mast cell–derived mediators (see reviews in references 4, 8, 9, and 23).

These findings show that, in WBB6F_1 mice, IgE-dependent mast cell degranulation can result in both reversible airway obstruction and airway hyperresponsiveness to cholinergic stimulation, in the absence of detectable infiltration of the airways with circulating leukocytes. On the other hand, it is now clear that eosinophil recruitment to the airways of mice can occur in response to aerosol challenge with antigen even in the virtual absence of mast cells, at least with some protocols of antigen sensitization and challenge. For example, in this issue, Takeda et al. (24) report that when Kit^W/$Kit^{W\text{-}v}$ and congenic +/+ mice were sensitized with OVA and then assessed 48 h after the last of 3 consecutive daily aerosol challenges with OVA, both the mast cell–deficient and the wild type mice exhibited similar numbers of eosinophils in bronchoalveolar lavage fluid and lung digests, as well as similar levels of airway hyperreactivity to methacholine challenge.

At least four previous studies (each using a different protocol of antigen sensitization and challenge, and, in some cases, a different antigen) also reported that mast cells are not essential for the development of antigen-induced infiltration of the airways with eosinophils (25–28). However, Kung et al. (27), using a protocol in which aerosol challenge with OVA was performed only twice on a single day, found that eosinophil infiltration of the airways in Kit^W/$Kit^{W\text{-}v}$ mice was ≤50% of that in the +/+ mice ($P < 0.05$) and was largely normalized after the selective repair of the animals' mast cell deficiency. Brusselle et al. (26), who performed daily OVA challenge for 7 d, also found that eosinophil influx into bronchoalveolar lavage fluid was reduced by ~50% in Kit^W/$Kit^{W\text{-}v}$ versus +/+ mice ($P = 0.06$).

Taken together, these five studies suggest that the relative contribution of mast cells to eosinophil infiltration of the airways may vary; mast cells may have no detectable role in experiments that use strong procedures of immunization and challenge, but may contribute significantly when protocols for sensitization and, especially, challenge have been selected to yield relatively attenuated responses. Moreover, in a model of peritoneal inflammation, the mast cell significantly enhanced the kinetics of leukocyte recruitment, even though it had no effect on the final magnitude of the response (29). The data of Kung et al. (27) indicate that mast cells can also enhance the kinetics of eosinophil responses to aerosol allergen challenge.

Takeda et al. (24) are the first to show that mast cell–deficient Kit^W/$Kit^{W\text{-}v}$ mice can express allergen-induced

airway hyperresponsiveness to cholinergic stimulation. This important observation provides yet more support for the now widespread view that there may be multiple routes to this defining characteristic of asthma. Indeed, it is thought that airway hyperresponsiveness, i.e., the development of bronchoconstriction in response to an immunologically nonspecific stimulus that would have no discernable effect in a normal individual, may reflect a consequence of any of a number of acute and/or chronic processes, including damage to the bronchial epithelium, submucosal edema, alterations of smooth muscle function (e.g., in response to mast cell mediators or other products present at sites of inflammation), and alterations in the production or degradation of neuroactive mediators (4, 8, 30). And although airway hyperresponsiveness and infiltration of the airways with eosinophils are often linked, both in animal models and in human asthma, airway hyperresponsiveness has been reported to occur in the absence of significant eosinophilia in certain settings such as in aerosol-challenged BALB/c mice that had been treated with an anti–IL-5 neutralizing antibody (31).

Depending on the model system, airway hyperresponsiveness also can occur either by IgE-dependent mechanisms (23, 32) or independently of IgE (33, 34) and/or IgG_1 (34). IL-5 derived from $CD4^+$ T cells has been implicated in the development of IL-4– and IgE/IgG_1–independent airway inflammation (34), adding to a large body of evidence that indicates that $CD4^+$ T cells can mediate airway hyperreactivity (35–37) as well as infiltration of the airways with eosinophils (25, 35–38). And although it has often been proposed that $CD4^+$ T cells promote the development of airway hyperreactivity indirectly through the recruitment of eosinophils and/or other leukocytes, the possibility that products derived from the T cells themselves can importantly contribute to airway hyperresponsiveness must also be considered (35).

Perhaps the simplest conclusions to draw from the various studies of mouse models of allergic asthma are that, in mice: (*a*) Airway hyperreactivity to cholinergic stimulation can occur by either mast cell–dependent mechanisms (which can be expressed even in the absence of leukocyte recruitment) or by $CD4^+$ T cell–dependent mechanisms (which typically occur in a setting that also includes eosinophil infiltration of the airways). (*b*) Mast cells are not necessary for the recruitment of Th2 cells or eosinophils to the airways after aerosol challenge with antigen, but can influence the kinetics or magnitude of the responses, especially at "suboptimal" levels of antigen exposure. (*c*) The extent to which eosinophils are necessary for the expression of T cell–dependent changes in airway hyperreactivity in different models remains to be fully defined. (*d*) In many experimental settings, particularly in various strains of normal mice, the expression of airway hyperresponsiveness (and other "asthma-like" features of these models) probably reflects the combined contributions of both mast cell– and T cell–dependent pathways.

But what about the role of the mast cell in "real" allergic asthma, in humans? It seems very likely that IgE-dependent mast cell activation importantly contributes to acute allergen-induced bronchoconstriction in human atopic asthma, and that mast cells can contribute to the airway inflammation associated with this disorder as well (2–4, 6, 8, 9). However, in humans, unlike in mice, the FcεRI can be expressed on several potential effector cells in addition to mast cells and basophils (14, 15). Also, the form of the FcεRI expressed on monocytes and dendritic cells (which lacks the β chain) can function to enhance the processing/presentation of antigens attached to proteins that are recognized by the cells' surface-bound IgE (14). Thus, in humans, IgE may not only serve to arm mast cells and other effectors of the efferent limb of acquired immune responses, but may also contribute, by promoting antigen processing/presentation, to the evolution of such responses.

In addition, two newly recognized aspects of FcεRI function or expression provide strong support for the hypothesis that mast cells (and perhaps other $FcεRI^+$ effector cells) may have a particularly important role in initiating and/or amplifying IgE-dependent inflammatory reactions, especially in response to low dose antigen challenge. First, Lin et al. (39) have identified the FcεRI β chain as an "amplifier" of signaling through this receptor, which can markedly upregulate the magnitude of the mediator release response to FcεRI aggregation; notably, it has been reported that certain mutations that result in amino acid substitutions in the human β chain may be linked to atopic disease (see reviews in reference 39). Second, studies in both mice (40, 41) and humans (42, 43) indicate that the level of expression of FcεRI on the surface of mast cells and basophils can be regulated by ambient concentrations of IgE and that this IgE-dependent upregulation of FcεRI expression both permits the cells to exhibit mediator release at lower concentrations of specific antigen (40, 42, 43), and also primes such cells to produce strikingly higher levels of certain mediators, including IL-4 and other cytokines (40, 43), under optimal conditions of antigen challenge.

These findings thus identify two FcεRI-dependent mechanisms (β chain "amplifier" function, IgE-dependent upregulation of FcεRI surface expression) for enhancing the sensitivity and intensity of the effector phase of IgE-dependent reactions. They also suggest a potential positive feedback mechanism ($\uparrow$IgE$\rightarrow\uparrow$FcεRI$\rightarrow\uparrow$antigen-, IgE-, and FcεRI-dependent release of IL-4 [40] and/or IL-13 [44]$\rightarrow\uparrow$IgE) by which mast cells (and possibly basophils) may enhance the further evolution, and persistence, of Th2-biased, IgE-associated immune responses. And studies in mice have identified yet another IgE-dependent, but apparently mast cell– and FcεRI-independent, mechanism to augment Th2 responses and associated eosinophil infiltration in the airways: IgE- and CD23-facilitated antigen presentation to T cells (28). Finally, mast cells and basophils may enhance IgE production via expression of the CD40 ligand (44, 45).

The clinical significance of these new findings largely remains to be established. However, this work clearly supports a complex, but more unified, view of the pathogenesis of allergic diseases, which proposes that both T cells and mast cells (and other $FcεRI^+$ cells) can have both effector

cell and immunoregulatory roles in these disorders. This hypothesis has a number of interesting implications with respect to existing, and proposed, therapeutic approaches for asthma and other allergic diseases. For example, anti–IgE-based strategies, which are already in clinical testing (42), not only may reduce CD23-dependent antigen presentation (28) and FcεRI$^+$ cell effector function (40–43), but also may diminish FcεRI$^+$ cell immunoregulatory function by reducing both mast cell (or basophil) IL-4/IL-13 production (40, 44) and FcεRI$^+$-dependent antigen presentation (14). Conversely, the findings that corticosteroids and other "immunosuppressive" drugs can diminish mast cell cytokine production, as well as reduce IgE- and mast cell-dependent inflammation and leukocyte recruitment in mice in vivo (see reviews in references 9, and 46), raise the possibility that the clinical benefits of such agents in asthma may reflect, at least in part, actions on mast cells as well as on the T cells, eosinophils, and other effector and target cells that participate in these complex disorders.

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The Mast Cell: A Versatile Effector Cell for a Challenging World

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Abstract
Mast cells are phenotypically and functionally versatile effector cells. When activated by IgE-dependent or other mechanisms, mast cells can produce a diverse array of mediators including TNF-α and many other cytokines. Moreover, mast cells can express increased numbers of high-affinity surface receptors for IgE (FcεRI) and enhanced levels of IgE-dependent mediator secretion in response to elevations in concentrations of IgE. These characteristics (and others) have suggested diverse *potential* roles for mast cells in health and disease. To test specific hypotheses about mast cell function in allergic reactions and other biological responses in vivo, one can employ genetically mast-cell-deficient Kit^{W}/Kit^{W-v} mice which do or do not contain adoptively transferred mast cell populations derived from genetically compatible wild-type mice or mice with mutations that influence mast cell biology. Such work has already indicated that mast cells (and, in some cases, mast-cell-derived cytokines) can have a critical role in the expression of the acute, late-phase and chronic components of IgE-dependent allergic inflammation and can influence the development of an important functional consequence of such reactions: airways hyperresponsiveness. However, mast cells can also perform important beneficial roles in host defense, both in IgE-dependent immune responses to certain parasites and in natural immunity to bacterial infection.

Introduction

It is a distinct honor and pleasure to have been asked to give the Paul Kallós Memorial Lecture and to have been provided with this special opportunity to discuss my favorite cell. Not only have I been fascinated with the mast cell for many years, but this cell has long been dear to many other members of the Collegium Internationale Allergologicum as well. Indeed, some would argue that the very existence of our organization, not to mention the entire specialty of allergy, depends on the clinical misbehavior of the mast cell, and its circulating 'counterpart', the basophil. However, evidence in the older literature, as well as compelling newer findings, indicates that mast cells (and perhaps even basophils) are not without significant redeeming value.

In this brief presentation, I hope to share some of my excitement about the mast cell, which is a wonderfully versatile effector cell and one which, depending on the circumstances, can serve us well or ill. By using the word versatile in this context, I wish to refer to several of the definitions of this term, including 'capable of doing many things competently', 'serving many functions,' and 'inconstant, variable or changeable' [1]. In arguing that all of these definitions apply to the mast cell, I will focus principally on findings derived from studies in murine systems. However, while the mouse and human systems may differ in details (some of them significant), I feel that most of the major principles regarding mast cell biology which have been derived from analyses of murine systems also apply (or, in all likelihood apply) in our species as well.

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Mast Cell Development and Heterogeneity: Plasticity and Variability of Mast Cell Phenotype

Although the first descriptions of mast cell heterogeneity (i.e. variation in aspects of mast cell phenotype in different anatomical sites in the same species) are at least 90 years old [2]; Enerbäck's [3] extensive analyses of the phenotypic heterogeneity of rat mast cells – many of which can be classified into two major categories: connective-tissue-type mast cells (CTMCs) and mucosal mast cells (MMCs) – helped to rekindle interest in this phenomenon. As pointed out by Enerbäck [3] and many others [4–6], the existence of distinct mast cell 'subtypes', particularly observations that different mast cells in the same species can vary in their mediator content and their susceptibility to various potential agonists of mediator release, suggested that phenotypically distinct mast cell populations might express different roles in health and disease.

However, despite many attractive and imaginative hypotheses [7], *proof* of the specific functions of mast cells, whether in disease or in normal physiology and immunology, had long remained elusive. Why was this? Even as recently as 20 years ago, there were still some quite significant technical and conceptual barriers to progress in understanding the development and specific functions of this cell type. For example, neither the origin of tissue mast cells, nor their growth factors and growth factor receptors, had been identified; there had been no description of methods to generate normal (nontransformed, nontumorigenic) mast cells in vitro in high purity; and there were no genetic approaches to investigate mast cell function in vivo, e.g. by comparing the features of biological responses elicited in the tissues of animals which did or did not contain mast cell populations. Happily for those working in this area, all of these issues have now been successfully addressed.

In 1978, Kitamura et al. [8] reported that WBB6F$_1$-*W/W^v* mice were profoundly deficient in tissue mast cells, but that these mice can develop tissue mast cells of donor origin after receiving transplants of bone marrow cells derived from either the congenic normal (+/+) mice or genetically compatible beige (C57BL/6-*bg/bg*) mice. This landmark study, and other work by Kitamura [9], firmly established the hematopoietic origin of tissue mast cells. However, the 'repair' of the mast cell deficiency of the *W/W^v* mice which was achieved by bone marrow transplantation was not specific in that the anemia of the recipient mice was also corrected, and other hematopoietic lineages were replaced by derivatives of the donor cells [8–11].

Building in part on Ginsburg's [12] seminal work in more complex tissue culture systems, as well as on Kitamura's identification of the hematopoietic origin of tissue mast cells, five groups independently reported in 1981 that cells with many features of mast cells could be generated in vitro from normal mouse hematopoietic cells maintained in certain T-cell- or WEHI-3-cell-conditioned media containing appropriate (but then undefined) growth factors [13–17]. The most important of such growth factors (in these systems) was later shown to be IL-3 [18]. Based on Ann Dvorak's ultrastructural analysis of our cells and other lines of evidence, we proposed that these mouse 'bone-marrow-derived cultured mast cells' (BMCMCs) represented immature mast cells which were committed to the mast cell lineage, but which might need additional signals (not present in the standard conditions of culture used at that time) to acquire phenotypic characteristics of mature CTMCs [13, 19].

In a confluence of the work in *W/W^v* mice and on the development of methods to generate lineage-committed mouse mast cells in vitro, Nakano et al. [20] analyzed *W/W^v* mice which had been injected with BMCMCs derived from the congenic normal (+/+) mice. This work showed that the injected BMCMC populations could acquire multiple phenotypic characteristics 'appropriate' for the anatomical site in which they resided. Thus, mast cells in the skin or peritoneal cavity gradually acquired biochemical, cytochemical and morphologic features of CTMCs, whereas those in the gastric mucosa exhibited features of MMCs [20]. In a subsequent study, Kanakura et al. [21] showed that clonal mouse mast cell populations derived from single peritoneal mast cells (i.e. clones derived from single CTMCs) could exhibit reversible and bidirectional changes in certain phenotypic characteristics between those characterized as 'CTMC-like' or 'MMC-like'.

However, it has been appreciated for some time, among those who like to classify rodent mast cells into 'subtypes', that two categories (CTMCs and MMCs) are simply not enough [3–6, 21–23]. This point has been illustrated nicely by Hunt and Stevens [22] with respect to mouse-mast-cell-associated proteases, and by Ide et al. [23] with respect to proteases in rat mast cells. Moreover, there is an ever-growing list of factors, including many cytokines, which can influence mast cell development and/or phenotype, at least in vitro [22, 24]. While this complexity may be confusing to the uninitiated, some general principles appear to be emerging, as summarized in table 1.

Table 1. Mast cell development and heteregeneity: general points

(1) Tissue mast cells are derived from committed c-kit+ precursors (e.g. the murine promastocyte) which arise in primary hematopoietic tissues and then circulate in the blood, but which mature in the peripheral tissues (some mature mast cells also occur in hematopoietic tissues).
(2) Mast cell numbers can increase because of increased recruitment/survival of mast cell precursors and/or as a result of proliferation of the more mature mast cells which are already present in the tissues.
(3) Mast cell populations in different anatomical sites, or even within individual tissues, may be comprised of 'subpopulations' of cells that exhibit variation in several aspects of phenotype and/or in their maturation or proliferative potential.
(4) The numbers and/or phenotypic characteristics of mast cells in individual tissues can change during immune responses, pathological processes and other biological responses, as determined by such factors as local concentrations of cytokines; at least some of these changes are potentially reversible.
(5) While the c-kit ligand, SCF, appears to be necessary for the survival and development of mast cells in normal tissues, many other cytokines (and other factors) can also influence mast cell development, phenotype and function.
(6) There are broad similarities in mast cell development, mediator content and function in murine rodents and humans, but there are also interesting and potentially important differences in mast cell biology among individual mammalian species.

Mast-Cell-Reconstituted Kit^{W}/Kit^{W-v} Mice: A General Model for Analyzing Mast Cell Function in vivo

In addition to providing a new approach for assessing the regulation of mast cell phenotypic heterogeneity in vivo, the report by Nakano et al. [20] also established a new method for studying mast cell function. In contrast to bone marrow infusion, the adoptive transfer of in-vitro-derived immature mast cell populations to W/W^{v} mice was achieved without altering the anemia of the recipient mice, indicating that the repair was selective for the animals' mast cell deficiency [20]. We therefore suggested that this general approach provided an opportunity to test hypotheses about normal mast cell function in animals whose tissues differed solely in either containing or virtually lacking mast cell populations of wild-type origin [20]. However, the same approach can also be used for analyzing the in vivo development or function of mast cells derived from mice with mutations that potentially influence various aspects of mast cell biology. For example, Nakano et al. [20] showed that in-vitro-derived BMCMCs of W/W^{v} mouse origin failed to survive after injection into W/W^{v} mice in vivo.

Table 2. General scheme for investigating mouse mast cell function in vivo

(1) Search for quantitative differences in the expression of the biological response in genetically mast-cell-deficient WBB6F$_1$-Kit^{W}/Kit^{W-v} and WCB6F$_1$-Mgf^{Sl}/Mgf^{Sl-d} mice and the congenic normal (+/+) mice. If the response is different in mast-cell-deficient and congenic normal mice, then:

(2) Compare[1] the responses in Kit^{W}/Kit^{W-v} and Kit^{W}/Kit^{W-v} mice that have received bone marrow transplantation from congenic (+/+) mice. Note: this determines whether the response which is abnormal is influenced by mast cells and/or other cells derived from hematopoietic precursors. If the abnormality in the response in Kit^{W}/Kit^{W-v} mice is 'corrected' after bone marrow transplantation from the congenic (+/+) mice then:

(3) Analyze[1,2] the response in Kit^{W}/Kit^{W-v} mice that have been selectively reconstituted with in-vitro-derived lineage-committed mast cells of congenic (+/+) origin ('mast cell knock-in-mice'). Note: this determines whether the response which is abnormally expressed in Kit^{W}/Kit^{W-v} mice has a mast-cell-dependent component. If mast cell reconstitution 'corrects' the abnormality in the response in Kit^{W}/Kit^{W-v} mice, then:

(4) Define the mechanism(s) by which mast cells contribute to the response. One approach would be to compare the expression of the response in Kit^{W}/Kit^{W-v} mice that have been reconstituted with normal mast cells as opposed to mast cells derived from mice with mutations that potentially influence specific aspects of mast cell function

[1] Appropriate studies should be done, as indicated for the particular project, to assess the numbers, phenotype and anatomical distribution of the mast cells which develop in the tissues of Kit^{W}/Kit^{W-v} recipients of donor bone marrow cells or mast cells; generally, several weeks are required for the mast cells to acquire phenotypic characteristics similar to those of the mast cells which occur in the same anatomical locations in normal mice. However, depending on the details of the experiment, the mast cells which develop in the Kit^{W}/Kit^{W-v} recipients may not be identical in number, anatomical distribution or phenotype to those in the same sites in the congenic (+/+) mice.

[2] Perform measurements to assess whether the Kit^{W}/Kit^{W-v} mice remained anemic after mast cell reconstitution in order to document the selectivity of mast cell reconstitution.

The general points to keep in mind when using BMCMC-injected Kit^{W}/Kit^{W-v} mice as a model to analyze mast cell function in vivo are presented in table 2 and reviewed in detail elsewhere [11]. Examples of how these animals can be used in the study of the roles of mast cells in IgE-dependent immune responses are given below.

The c-Kit Ligand (Stem Cell Factor): A Major Regulator of Mast Cell Development, Survival and Function

As reviewed at our CIA meeting in Nantucket [25] and elsewhere [24], the ability of W/W^v mouse tissues to induce mast cell development in normal but not W/W^v hematopoietic cells or mast cells is now understood in molecular terms and primarily reflects the interaction between the c-kit receptor (which is expressed on early hematopoietic progenitor cells, mast cells, melanocytes, germ cells and certain other cell types) and the c-kit ligand stem cell factor (SCF) [26, 27], (also known as mast cell growth factor, (MGF) [28], kit ligand (KL) [29] and steel factor [30]. c-Kit is expressed on both mast cells and their precursors, including the earliest committed mast cell precursor to be identified in the mouse fetal circulation, the promastocyte [31], but is functionally impaired in W/W^v mice [32, 33]. SCF, which can be expressed in membrane-associated or soluble forms by many cell types, is now known to represent a major survival and developmental factor for both murine and human mast cells [24, 25, 34]. Now that the *W* locus has been identified as encoding c-*kit*, and the *Sl* locus has been identified as allelic with the gene for MGF (or SCF), the proper designation of genetically mast cell-deficient W/W^v or Sl/Sl^d mice is Kit^W/Kit^{W-v} or Mgf^{Sl}/Mgf^{Sl-d} mice, respectively [35].

While the effects of SCF on mast cell development were anticipated, in light of the virtual absence of mature mast cells in Kit^W/Kit^{W-v} or Mgf^{Sl}/Mgf^{Sl-d} mice, the profoundly mast-cell-deficient phenotypes of the *Kit* or *Mgf*-mutant mice did not suggest another important, but unexpected, consequence of the interaction between SCF and its receptor – the modulation of mast cell function. However, it is now clear that under some circumstances SCF can promote c-*kit*-dependent mast cell degranulation and mediator release [36–39], as well as enhance the release of mast cell mediators via FcεRI-dependent mechanisms [37, 39, 40]. Furthermore, SCF-dependent promotion of secretory functions in cells which express c-kit is apparently not restricted to the mast cell lineage, as some human subjects who received recombinant human SCF in phase I trials developed persistent cutaneous hypermelanosis and melanocytic hyperplasia, as well as acute degranulation of dermal mast cells, at sites injected subcutaneously with recombinant human SCF [34].

The finding that SCF can regulate mast cell mediator production, as well as mast cell survival and development, has a number of potentially important implications for our understanding of mast cell function [24, 34, 41]. For example, in certain mouse mast cell populations, SCF can promote mast cells to release IL-6 (and to a lesser extent, TNF-α) under conditions which result in little or no detectable release of biogenic amines [42]. It will be of interest to assess whether such 'differential' release of mast-cell-derived cytokines by SCF might hold keys for understanding mast cell function during some pathologic processes or even in normal physiology [24, 42].

Mast Cells and Mast Cell-Leukocyte Cytokine Cascades in Allergic Inflammation, Immunologically Nonspecific Inflammation and Host Defense

Mast-cell-reconstituted Kit^W/Kit^{W-v} mice, which we also refer to as 'mast cell knock-in mice', provide a useful model system for characterizing mast cell function during IgE-dependent immune responses in vivo and can also be helpful as part of studies to identify the potential mediators by which mast cells might express such functions. For example, our initial studies of IgE-dependent cutaneous inflammation in mast-cell-deficient Kit^W/Kit^{W-v} and congenic normal (+/+) mice and in mast-cell-reconstituted Kit^W/Kit^{W-v} mice revealed that essentially *all* of the tissue swelling, local extravasation of ^{125}I-fibrinogen (a measure of increased vascular permeability) and deposition of cross-linked ^{125}I-fibrin (indicative of local activation of interstitial clotting) induced by intravenous antigen challenge in mice which had been injected intradermally with IgE was mast cell dependent [43]. We then showed that essentially all of the leukocyte infiltration associated with IgE-dependent cutaneous inflammation in the mouse was also mast cell dependent [44].

Although there were several candidate mast-cell-associated mediators which might contribute to mast-cell-dependent leukocyte recruitment in this setting, we were particularly interested in TNF-α. In a series of studies [45–47], we had identified mouse mast cells as a potential source of TNF-α, and showed that this cytokine could be released from the cells via both immunologically specific and immunologically non-specific mechanisms. Moreover, certain mouse mast cells had stores of TNF-α that were associated with the cytoplasmic granules and which could be released rapidly upon appropriate stimulation, along with a more sustained release of TNF-α derived from a newly synthesized pool [45–47].

In tests in normal mice, we found that IgE and mast-cell-dependent leukocyte infiltration could be inhibited by approximately 50% with a neutralizing antibody to TNF-α

[44]. Although, by this time, mast cells had been defined as a source of many different cytokines with diverse potential activities [48–51], this study provided in vivo evidence that at least one of these mast-cell-associated cytokines (TNF-α) actually could contribute to a defined mast cell function in vivo – the recruitment of leukocytes at sites of IgE-dependent immune responses [44]. Subsequently, Gordon and Galli [52] used mast cell knock-in Kit^{W}/Kit^{W-v} mice to show that these same IgE-dependent skin reactions can be associated with a marked, transient and entirely mast-cell-dependent local upregulation of dermal fibroblast type I collagen mRNA expression. We further demonstrated, using in vitro approaches, that both mast-cell-derived TNF-α and TGF-β can contribute to mast-cell-dependent induction of fibroblast collagen gene expression.

The emerging idea that mast cell cytokine production may significantly contribute to some of the characteristic acute, late-phase and chronic components of allergic inflammation clearly has implications for the therapy of allergic disorders. In support of this concept, Wershil et al. [53] demonstrated that either dexamethasone or cyclosporin A could suppress the mast-cell-dependent tissue swelling and/or leukocyte infiltration associated with IgE-dependent cutaneous reactions in mice, and they reported in vitro evidence that these effects of the drugs may in part reflect their ability to inhibit mast cell TNF-α production [53, 54] and in part other actions, such as suppression of the effects of TNF-α on local target cells [53].

Taken together, these and many other lines of evidence indicate that TNF-α and other cytokines of mast cell origin represent potentially important (and therapeutically accessible) links between mast cell activation and the leukocyte infiltration and tissue remodeling associated with chronic allergic disorders [51, 55–57]. However, it should be emphasized that many other biological responses not thought to involve IgE may also be importantly influenced by mast cells and their cytokines (and other mast-cell-derived products), including examples of both immunologically specific and immunologically nonspecific inflammation. For example, we showed that when PMA was applied to the mast-cell-reconstituted or contralateral mast-cell-deficient ears of Kit^{W}/Kit^{W-v} mice, both the ensuing tissue swelling and leukocyte infiltration were significantly greater in the presence than in the absence of mast cells [58]. And Qureshi and Jakschik [59] used mast-cell-reconstituted mice to show that mast cells can also contribute to the leukocyte recruitment induced by the intraperitoneal injection of thioglycollate.

What are the general implications of such studies? It has long been believed that immunologically nonspecific acute inflammatory responses can be a critical component of natural immunity to infection with bacteria and other pathogens. Given their ability to release mediators of inflammation in response to activation by many immunologically nonspecific stimuli [3–7, 56, 57] and in light of their strategic anatomical distribution near surfaces exposed to the external environment [3–7, 10], mast cells are very well suited to function as sentinels of innate immunity. One key facet of the mast cell's function in this setting may be to initiate and orchestrate the acute inflammation which represents an important part of the early host response to microbial infection. In support of this hypothesis, two groups have now demonstrated, using mast-cell-reconstituted Kit^{W}/Kit^{W-v} mice, that mast cells and mast-cell-derived TNF-α can indeed have a significant protective role in two different models of natural immunity to bacterial infection [60, 61].

Thus, it appears that the mast cell can be a remarkably versatile effector cell in health and disease. Even if we restrict our focus to the roles of mast cells in inflammation and tissue remodeling, which is probably much too narrow a view of the many potentially important activities of this cell, several lines of evidence indicate that the mast cell can have either adaptive or harmful functions when activated via IgE or by other mechanisms [62]. It can promote immunologically nonspecific inflammation, which under some circumstances may result in pathology, but in other settings can contribute to natural immunity to certain bacterial infections. It can also orchestrate IgE-dependent responses. These can be protective in IgE-dependent resistance to certain parasites, as illustrated by Matsuda et al. [63] with respect to reactions to the cutaneous feeding of larval *Haemaphysalis longicornis* ticks. But IgE and mast-cell-dependent inflammation can also be harmful, especially if the eliciting stimulus is essentially innocuous, as is typically the case in allergic diseases.

However, it should be kept in mind that natural selection has engineered considerable redundancy into the effector mechanisms which contribute to the success of host defense. The relative importance of mast cells versus basophils in IgE-associated immune responses may constitute a particularly relevant example of this point. While the preponderance of evidence indicates that circulating basophils are granulocytes that are distinct from the mast cell lineage, these two cells probably express some highly overlapping functions [56, 64]. In certain acquired immune responses to the feeding of larval ticks, mast cells appear to be essential for the successful expression of host resistance [63]; by contrast, in other tick responses, basophils may be as (or even more) important effector cells than mast cells [65, 66]. The practical point, either for the analysis of host resistance

or allergic inflammation, is that specific functions of mast cells (and other effector cells) may be revealed under certain experimental conditions, but obscured by others, particularly if the response is one characterized by extensive redundancy in effector mechanisms.

The Regulation of Mast Cell and Basophil FcεRI Expression by Circulating IgE

Now that the expression of biological responses can be compared in the tissues of mice which either contain or virtually lack mast cell populations, it is becoming clear that mast cells can be essential for many of the important acute, late phase and perhaps even chronic features of IgE-dependent immune responses, at least in mice. Mast cells are also largely (if not entirely) responsible for the bronchial hyperresponsiveness to methacholine challenge which can be detected in mice shortly after their intravenous challenge with anti-IgE antibodies [67], a finding which supports the hypothesis that mast cell products may account, at least in part, for the bronchial hyperreactivity which is so characteristic of human asthma.

Mast cells thus can have diverse functions which regulate the local expression, and consequences, of IgE-dependent immune responses. However, it now appears that one important aspect of the versatility of mast cells as effector cells in IgE-dependent immune reactions has not been fully appreciated. Almost 20 years ago, two groups independently demonstrated a strong positive correlation between serum IgE levels and numbers of IgE molecules (and numbers of FcεRI) expressed on human basophils [68–70]. While the authors favored the hypothesis that basophil receptor number is modulated by the serum IgE concentration [70], other possibilities, such as the regulation of both FcεRI and serum IgE by the same cytokines (or other factors), had not been ruled out. We therefore took advantage of the murine system to examine the effect of IgE concentrations on mouse mast cell and basophil FcεRI expression.

We found that genetically IgE-deficient mice exhibit dramatically (80%) reduced peritoneal mast cell FcεRI expression compared to the corresponding normal mice [71], and that exposure to monoclonal mouse IgE results in a striking (up to 32-fold) upregulation of surface expression of FcεRI on mouse mast cells, both in vitro and in vivo [71]. Administration of IgE in vivo also results in a striking elevation of mouse bone marrow basophil FcεRI expression, as detected by flow cytometric measurements of IgE binding by these cells [72]. Finally, we found that the IgE-dependent upregulation of FcεRI expression can significantly enhance mast cell serotonin and cytokine release through effects on both the sensitivity and intensity of the response [71]. Similar findings also were obtained with in-vitro-derived human mast cells [73].

These observations, when taken together with recent work with human basophils by Lichtenstein [74], strongly suggest that mechanisms which result in substantial elevation of the levels of circulating IgE may also result in enhanced IgE- and FcεRI-dependent effector cell function, both through effects on the sensitivity and intensity of the cells' IgE-dependent secretory response to a single antigen and by permitting the cells to be effectively and simultaneously sensitized to larger numbers of antigens. In acquired immune responses to parasites, this process would be expected to benefit the host. Unfortunately, the same mechanism would also increase the severity of allergic disease.

Conclusion

The recognition that mast cells represent sources of TNF-α and many other cytokines, which have in aggregate an enormous array of *potential* roles in physiology, immunology and pathology, together with the development of new animal models which permit hypotheses about mast cell function to be definitively tested in vivo, has provided new insights into the roles of mast cells in health and disease. It is becoming increasingly clear that mast cells can contribute not only to the acute manifestations of IgE-associated allergic disorders, but also to the leukocyte infiltration and tissue remodeling which importantly contribute to the pathology associated with some of these diseases. Moreover, the mast-cell-dependent aspects of these disorders represent potentially attractive targets for therapeutic intervention.

In studies of host defense, mast-cell-reconstituted Kit^{W}/Kit^{W-v} mice have been used to show that mast cells can contribute significantly to acquired resistance to some parasites and to natural resistance to certain bacteria, at least in mice. Indeed, patients with allergic disorders may be paying an unfortunate price for the development, over the long span of vertebrate evolution, of a system of host defense that not only generates antibodies against foreign substances in the environment (such as those derived from parasites), but which has equipped the mast cell to function as a strategically located, highly reactive and very potent effector cell in host responses to invading pathogens. And even though allergists and immunologists most readily think of mast cells in the context of IgE-dependent immune responses, it seems increasingly likely that mast cell function in some examples

of host defense, and in certain disease processes, may reflect patterns of mast cell mediator release which differ from those observed in cells which have been acutely activated via the FcεRI.

Finally, it is now evident that mast cell populations, and even individual mast cells, must be considered to represent dynamic and plastic effector cells which, depending on the circumstances, can exhibit significant variation in such important aspects of phenotype as mediator content, susceptibility to activation by nonimmunological or immunological stimuli of activation and level of expression of FcεRI.

All of these new findings have not only been enlightening in their own right, but have encouraged us to expect much additional progress in our understanding of this versatile and fascinating, but increasingly less enigmatic cell.

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